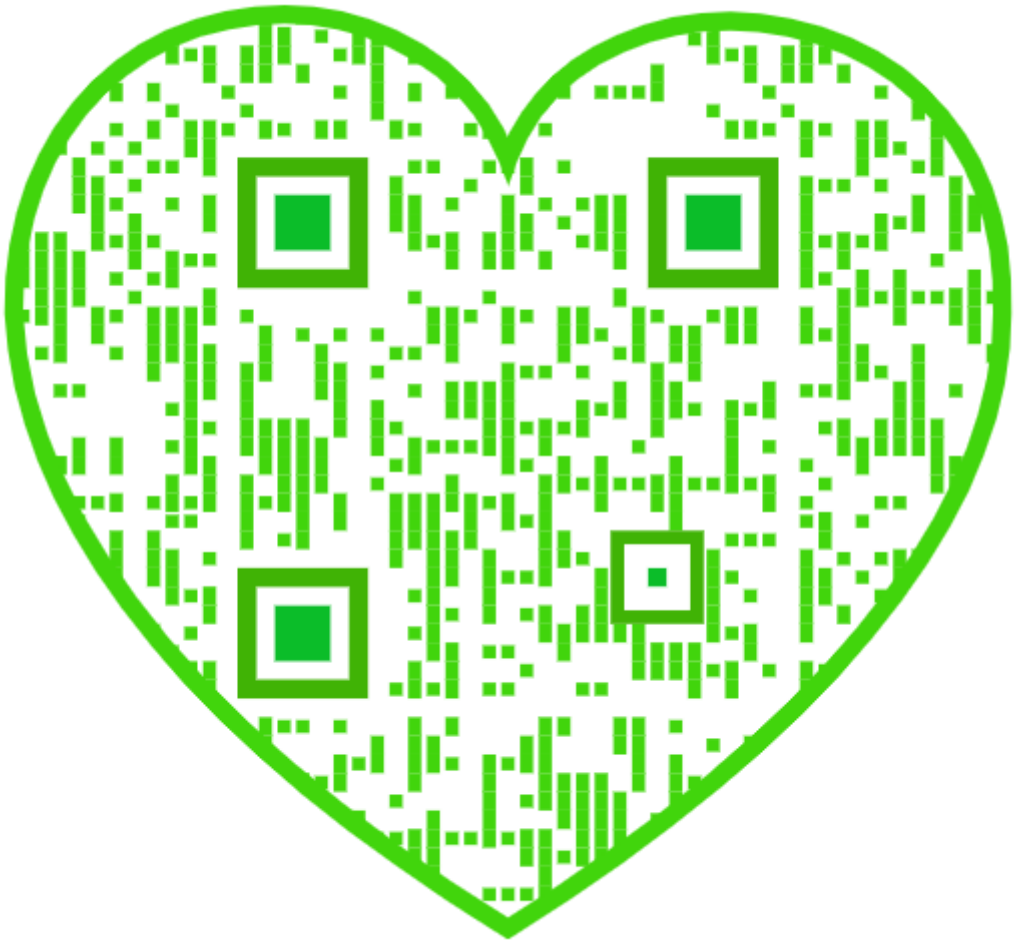

LABORATORY GUIDE FOR HISTOLOGY

HARDESTY

A LABORATORY GUIDE FOR HISTOLOGY

Laboratory Outlines for the Study of Histology and Microscopic Anatomy

BY

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WITH A CHAPTER ON LABORATORY DRAWING BY

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WITH 30 ILLUSTRATIONS 2 OF WHICH ARE IN COLORS

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PREFACE.

THE purpose of this little book is economy of time and labor for both the Instructor and Student. It is a common experience in our Anatomical Laboratories that the student taking courses in Histology and Microscopic Organology spends a large part of his time in doing things which are not essential to the accomplishment of the actual purpose of the courses. He wastes time in vague application of histological technic which is involved in neither the title nor the real purport of the work. He proceeds to study a subject without a definite concept of what is best to be done and how best to go about it, and without forethought of economic distribution and use of his time. He will begin a drawing from a preparation without having first decided what special features the drawing should most advisably illustrate, without having thought out the amount of magnification and the style of drawing most suited to the purpose. The too frequent consequence is that the time allotted to a course is so poorly utilized that the student actually covers a far smaller portion of the ground purported to be the object of the course than he may be led to cover if guided in a more definite manner as to what to do and how to proceed.

This Laboratory Guide, therefore, is offered to the student and instructor with the hope that it will be an aid toward the accomplishment of a greater amount of that work which may be correctly designated The Study of Histology and Microscopic Anatomy, and an aid, further, toward an improvement in the quality of that work. On the other hand, it is not intended that it shall, in any way, lessen the tendency to nor decrease the amount of independent work on the part of the student as regards all the essential features of his studies. Rather, the suggestion and stimulation of independent thought has been kept in mind throughout the construction of the guide.

The Outlines as here given embody what the author has used with his classes during the past six years. Corrected and revised from year to year, they have heretofore been issued to the student in mimeographed form and, in their revision, restriction has been more and more made to those studies deemed most essential and to the amount of work which experience has shown that the average class in Histology may be induced to cover in the allotted time.

As offered by the author, the entire work has required the equivalent of three three-hour periods per week throughout one school year. The time has been so arranged as to offer the work in three courses: (1) Histology proper; (2) Microscopic Anatomy of the Organs, exclusive of the Central Nervous System and Sense-Organs; (3) The Central Nervous System and

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Organs of Special Sense. In medical schools, the third course is more advisedly given to students in the second year when they are more mature from experiences in other courses and capable of more rapid and intelligent accomplishment. Unfortunately, some of the schools in which it is hoped this Laboratory Guide will be used do not devote to the work as many hours as are necessary to the amount of work outlined here. In such cases, if the time cannot be increased, it will be necessary, of course, that the instructor read ahead of his class and direct that such paragraphs as he deems less essential be omitted. It should be remembered, however, that certain of the time called for here is devoted to studies of the detailed gross anatomy of the organs, not only for the value of such studies *per se*, but in order to bridge that too common gap between Gross and Microscopic Anatomy, and that, therefore, the whole time is not demanded for purely microscopic structures. Especially is this true of the portion dealing with the central nervous system, which portion is intended to comprise the only study made by the student of the gross anatomy of this system.

It is a common experience that the student's laboratory drawings in Histology are a matter of troublesome as well important necessity. In order that the student, so often wholly unskilled in drawing, may obtain some idea of the simpler principles involved in graphic art, Dr. A. W. Lee has kindly prepared a chapter on laboratory drawing. This Laboratory Guide is especially fortunate in its incorporation of this chapter, for it is a subject in which systematic instruction is very seldom given, and not only is Dr. Lee himself an artist of marked ability, but he has given considerable study to this variety of drawing. The chapter involves directions as to equipment and for such procedures and processes as are considered most essential for the student in the laboratory. The original drawings illustrating the chapter are purposely reproduced here without reduction that they may be more instructive as to the direct effects the student may accomplish in his laboratory drawings.

Berkeley, California, August, 1908. I. H.

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LABORATORY GUIDE FOR HISTOLOGY.

SECTION I.

LABORATORY DRAWING.

DRAWING of microscopical preparations has become such an integral part of the routine class-work in histology and microscopic organology, that it is thought advisable to include in this Laboratory Guide a brief description of certain of the materials and the methods used to express upon paper the structures revealed by the microscope. The prices given under the description of the respective articles may vary somewhat with the locality, but on the whole may be taken as quite approximate.

Technical art terms, even those that have become part of nearly everyone's vocabulary, have likewise been avoided in order that the clearness of description might not be obscured in any manner. The individual who "can't draw" has constantly been kept in mind; in fact, this chapter was undertaken solely for his benefit. It is quite true that as yet no formula has been given which can supply what Nature has not endowed, or, to put this idea in other words, no one can learn to draw simply by reading a list of instructions. But it is, nevertheless, a fact that the ungifted will find his task easier if he has been told how to go about it. The following pages will attempt to do that much.

DRAWING MATERIALS.

These may be described under two heads: those used in making black and white drawings and those employed when colors are necessary.

The articles common to both black and white drawing and drawing in color will be considered first. These are, drawing-board, hand-rest, thumb-tacks, papers, pencils, erasers, pens, pen holders, black pigments and mixing-dish.

Drawing-board.—The use of the mere surface of the work-table for drawing, frequently practiced in laboratories, is not conducive to the best results. Not only is the wood of the table often not adapted to the necessary use of thumb-tacks for holding the drawing-paper in place, but the continued use of the thumb-tacks results in a needless disfiguring of the table. Furthermore, the table top does not allow the conveniences of work upon an inclined plane, when desired, nor manipulation of the position of the fixed drawing-paper while at work, and, being used in common for the reagents and other materials employed in the work, soiling of the paper often results. The use of a small

drawing-board is advised. There is no need to buy this, as a very serviceable one can be made from any piece of unwarped, smooth, soft wood. It should be cut about 8 inches wide by 12 inches long. Boards of greater dimensions than these should be avoided, because of the unused space they occupy and their clumsiness in handling and use with the microscope. Flush with one end of the board, and on its under side, a thin strip of wood may be nailed; that is, if the operator cares to work constantly upon an inclined plane. If a flat surface is preferred, the strip, of course, will be omitted, or, for occasional use, need not be attached to the board. The softness of the wood permits an easy insertion and extraction of thumb-tacks, and its consequent lightness facilitates

FIG. 1.—Illustrating the use of the drawing-board, *b*, and hand-rest, *a*, and the proper position for a right-handed person when drawing microscopical preparations. The raised end of the hand-rest *a*, is placed upon the drawing-board *b*, and its other end allowed to rest on the table.

manipulation during the process of drawing, for, it must be borne in mind, that a slight rotation of the paper this way or that often makes it possible to put in certain lines with a sureness and truthfulness which could not be accomplished if the drawing were immovably fixed to the work-table.

Hand-rest.—This can easily be dispensed with. If it is desired, it should also be made of light, smooth wood. The hand-rest is such a simple contrivance that anyone can construct it. It merely consists of a piece of thin, rigid wood some 12 inches long by 4 inches wide, and a thin strip, say $\frac{3}{4}$-inch square, nailed flush with one end on its under side. The object is, as the name implies, to furnish a support for the hand, it often being noticed that the fine details are better executed when the hand is raised and steadied some distance above the paper than when down, close upon it. Again, the hand-

rest aids to keep the drawing clean, since contact of the naturally moist skin with the clear white surface frequently leaves a stain, especially if the drawing is being done upon some rough, absorbent material, such as Ross board. Also, the use of a hand-rest often obviates a cramping of the fingers in long-continued drawing. The manner of using both the drawing-board and hand-rest is shown in Fig. 1.

Thumb-tacks.—Fig. 2 gives three styles of thumb-tacks, *D*, *E*, and *F*. The first one is by far the best. It has a solid cap over the head, and can be obtained in the size pictured, either brass or nickel. *E* illustrates a variety in which the whole tack is made of one piece, the point being punched out of the head and turned down. The one objection to it is that after some use the point either bends or cracks off at its junction with the head. *F* shows a very unsatisfactory style, which should never be selected if the other varieties can be obtained. The fixed end of the point passes through and presents in the surface of the head. Often but limited use suffices to loosen the point, and on some occasion, when being pushed home into the drawing-board, will spring clear of the head, and a painful prick of the finger may result. The price of *E* and *F* is about the same as that of *D*.

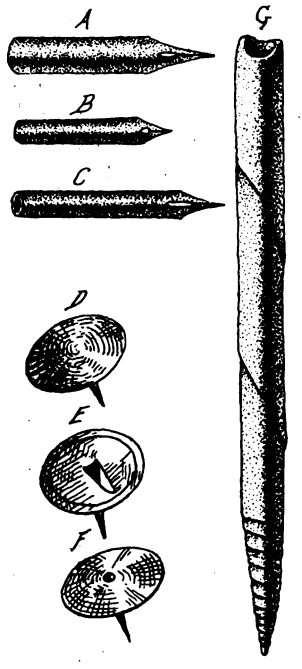

FIG. 2.—Showing styles of thumb-tacks, drawing pens and a style of one of the cheaper and commonly used "blenders" for pencil drawing.

Drawing-papers.—Under this head the so-called drawing-boards are also included. The vast variety of these papers excludes in these pages anything like a complete list of them. Some attention will be paid to certain of those in more common use.

Whatman's water-color paper is made in a number of different surfaces, but the one termed "hot-pressed" is the only kind serviceable for ordinary microscopical illustration. It is a paper of beautiful whiteness and just rough enough to take easily the graphite of the pencil. It, as well, is equally adapted to the use of fluid pigments. A heavy hand, however, finds this paper not as satisfactory as the boards, since its softness often leads to a sticking of the pen with consequent blotting. This paper can be obtained in different sizes and thicknesses, but 2-ply Imperial will be found quite convenient for all illustration, as this term is here used Hot-pressed, 2-ply imperial is furnished at the rate of two sheets for 25 cents.

If only one kind of drawing-paper were to be recommended, perhaps none would be so generally satisfactory as that known as *Reynolds' bristol board*. It is of pure and permanent whiteness and of extremely hard surface and body.

This paper, therefore, suffers less from "knife-erasures" than a softer materia would and, because of this fact, is especially to be advised for student work. For pen-and-ink drawings, and even for those done in masses of fluid pigment, there is nothing better to be had. But in pencil drawings its somewhat smooth surface makes it not as satisfactory for the unskilled hand as some of the rougher papers, since the graphite of the pencil is not so easily taken as by the latter. This one unpleasant feature, however, should not discourage a trial of such an otherwise excellent paper even for pencil-work. It is sold in different sizes and thicknesses, but sheets 12½ inches by 15 inches and of moderate weight can be had under the name of "Cap 2-ply" for 90 cents per dozen.

Examiner board is much used because of its very appropriate surface for pencil drawing. Also pen and ink give with it equally good results. It is not pure white, however, leaning more to a pinkish-cream color. It is unsatisfactory when "knife-erasures" have to be made, as its surface and body are somewhat soft. In the Imperial size it is sold at the rate of two sheets for 25 cents.

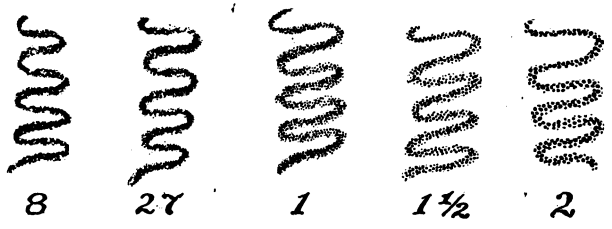

FIG. 3.—The result of drawing a pencil-point without raising it from the surface, over five varieties of Ross Board. The number given under each corresponds to the list-number by which the style is designated.

The *Strathmore papers* are also of great service. The bristol board of this make is sold in sheets measuring 23 x 29 inches at the rate of two for 25 cents.

Whatman's water-color paper, Reynolds', Examiner and Strathmore bristol boards consist throughout of but one material, paper-pulp, and, therefore, their working-surface is paper, whether smooth or rough. But there is another board on the market, which consists of a chalk surface on a paper body. This is known as *stipple board* and is manufactured by the Chas. J. Ross Company. The surface of this paper is made in a generous assortment of patterns suited to different degrees of stipple effects and adapted to different purposes in reproduction, but for drawing in microscopic anatomy not more than six of them are applicable.

The patterns are listed and referred to by numbers, so when ordering one asks for Ross stipple board No. 1, 1½, 2, 27, etc. For most work Nos. 1 and 27 will be used, but there are instances where 1½, 2 and even other patterns

are very useful. No. 8, for example, is useful for drawings in which considerable perspective is demanded. The advantage of this paper in microscopical drawing is that a great number of dots can be made in a surprisingly short time, simply by rubbing a pencil back and forth over its surface. Results produced with pencil on five varieties of the board are shown in Fig. 3. Subsequent scratching out of these dots to represent either uncolored elements in the preparation, or places in it where the transmitted light comes through clefts in the tissues, will give remarkably realistic effects. Scratching is effected with great ease and accuracy since it merely consists in abrasion of the chalk surface of the board. Lines and shadows may be strengthened with the pen, and at need it takes wash excellently. A later figure (Fig. 5) is given illustrating the use of Ross board. Unfortunately, it is expensive. The half-sheets are usually retailed for 30 cents and the full sheets for 50 cents, and neither of these is very large.

Pencils.—There is hardly a limit to the number of pencils which can be used to advantage, but for all practical purposes three of varying degrees of softness will suffice. Again, several firms manufacture pencils of repute, but for the sake of simplicity, there need be mentioned here only the well-known drawing pencils made by Faber and the Koh-I-Noor, made by L. & C. Hardtmuth in Austria. The grade known as 4H will be found hard enough for the finest and lightest of lines. HB is a good grade for the placing of outlines, while 5B is as soft and black as usual work will demand for the laying of massed color. Even in cases demanding extreme hardness, the 6H will be sufficient. These pencils are usually sold at 10 cents straight.

Erasers.—These are divided into two classes, one for pencil and one for pen-work. A casual glance at the supply of these in any well-stocked art store is sufficient to bewilder the beginner. But one suggestion will be of service in selecting a pencil-eraser: Do not purchase a hard, colored article. Hard erasers destroy the surface of the drawing-paper and colored ones, if used vigorously, are very apt to leave their own tint upon the paper. The so-called "ink-erasers" had also better be avoided entirely, as they either fail to work at all or they sadly abuse the surface of the material drawn upon. Any small, well-sharpened knife-blade, gradually and carefully applied, is much more a tool of choice in the removal of ink than the ready-made erasers. Good pencil-erasers are made by Hardtmuth, stamped with a large H and should not cost more than 10 cents at the most. Any eraser chosen should be soft and should wear rapidly when applied to the paper.

Pens.—Here again one meets with a well nigh infinite assortment. Still, two pens of long-established worth will meet all the demands laid upon the beginner, and are described as follows: Joseph Gillott's Lithographic Pen, No. 290, is one of very fine point and, for placing delicate lines, it will give entire satisfaction. Its point, however, is so flexible that an unsteady hand often robs it of its best work. Gillott's Lithographic Crow Quill, No. 659, is of more rigidity and, in consequence, the beginner will do better line-drawing with it

than with No. 290. For stippling, that is, the placing of dots in close proximity, this Crow Quill pen is so excellent that hardly another should be named with it in the same class. These pens are illustrated in Figure 2, A and C, respectively. A third pen, made by the same firm and known as the Superfine Drawing Pen, No. 1000, is pictured in Figure 2, B, and gives, with a steady, light hand, the most delicate lines conceivable. But since it is many times more sensitive than No. 290, it should not be used unless the operator is quite sure of his touch. The cost of these pens is as follows: Nos. 290 and 659, 50 cents a dozen, while a card of 12 points, No. 1000, is sold for $1.00. The Keuffel Esser Co. also makes a drawing-pen, known as Lithographic Pen No. 3205, with which very fine lines can be made and it may be used with especially good results in stipling.

Pen Holders.—The four varieties of pens above named each include, on the same card with the points, a pen holder especially adapted for the respective pen, which, if properly used, will be all that one needs. Still, the thinness of these holders often leads to a cramping of the beginner's fingers. To obviate this, one of the many styles of cork cylinders may be slipped over the holder, thus increasing its girth. Or, a holder closer resembling that of the ordinary writing-pen may be obtained for 10 cents or 15 cents. This matter of having a thick pen holder should be considered seriously, for many instances are met where individuals are able to do practically nothing with the thin tool, but who render quite satisfactory drawings with one of greater girth.

PIGMENTS.

Black Pigment.—This may be a fluid, as in the ordinary drawing-inks and aqueous mixtures of a solid color; or in a dry state, either as a powder or in mass. Graphite-filings and lamp-black are examples of powdered black pigment, and the ordinary water-color blocks and certain of the crayons represent the same pigment in mass.

Of the ready-mixed black drawing fluids, those made by Chas. M. Higgins & Co. will be found very satisfactory. They are to be had in two varieties, one which will permit a superimposing of watery solutions upon them and known as waterproof, and another which would run if so treated, the non-waterproof or general drawing ink. Since there is so little choice between these two, that is, so far as their drawing qualities are concerned, one had better buy the first-named kind, as its waterproof character is often of distinct advantage. As manufactured at present, this ink has a white label; the general or non-waterproof variety being furnished with one of red. Both kinds sell at 25 cents a bottle. Higgins' ink can hardly be surpassed when used in full strength, but upon dilution it shows a brownish rather than a blackish tint.

Where dilution of a drawing-fluid is desired, Winsor and Newton's Process Black, coming in receptacles of about 1 oz. capacity, will be found to give very true intensities of black. It is sold for 25 cents a bottle.

Graphite-filings are to be made as directed under the remarks on black and white drawing.

Occasionally lamp-black can be used, but its tendency to show a murky-brown upon thin distribution over the drawing-paper often leads to an unpleasant result, for which reason the beginner, should occasion arise for use of powdered pigments, is advised to confine his work to the graphite filings.

Of the mass pigments, Winsor & Newton's Ivory Black offers as many virtues as any other and is sold for about 25 cents a cake.

Mixing-trays may be dispensed with if one does not desire to mix his own drawing fluid. However, if this be done, a porcelain dish of four compartments will give excellent service. It should not cost more than 25 cents.

The Tortillion, or "blender," is a tool practically confined to pencil drawing and is pictured in Fig. 2, G. It is a sort of rod, constructed of spirally-wound paper and comes in two varieties, gray and white, each costing about the

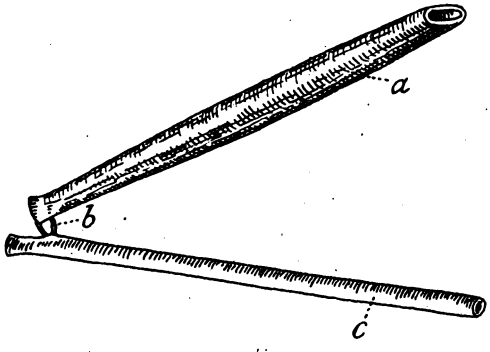

FIG. 4.—A drawing of the style of atomizer used in fixing drawings.

same, 60 cents a gross. Special information concerning the tortillion's use can be found in the treatment of black and white drawing. Tools of similar purpose, called stomps, but constructed of different materials, such as leather, are also on the market. Still, these are far too large, as a rule, for any ordinary laboratory work, and besides cost considerably more than the paper articles.

Fixing is a process used only where the applied pigments have a tendency to rub off or blend in handling, such as graphite and lamp-black. Two articles are necessary—a fixing solution and an atomizer. The former is simply a thin solution of a resin (Mastic, shellac, damar, etc.) and can be obtained in various quantities, a 2-oz. bottle costing about 15 cents.

The atomizer consists of two tubes of japanned iron, *a* and *c*, Fig. 4, linked together by the hinge *b*. The tube *a* is flattened from above downward at its free end, so that it may be held conveniently between the lips. The tube *c* is cylindrical and is the one whose distal end is immersed in the fixing solution. Directions for the use of both of these articles can be found in the remarks on Fixing (page 23).

COLORS.

There are three types of material used in making colored drawings, namely, crayons, water-colors and colored drawing inks. These present an astounding variety and accordingly cannot be discussed in detail.

Crayons.—A. W. Faber's colored crayons are entirely reliable, and so much more can be done with them than with the cheaper makes that they are the only ones here described. The box containing 8 colors costs 75 cents, but the difference in price between it and the set of twelve is not of sufficient advantage to make up for the loss in assortment of tints. The buyer may compose his outfit according to his own choice of colors, but the following assortment will be found sufficient for all general purposes:

Black	Green Bice	India Brown, No. 1
Prussian Blue	Deep Chrome	India Brown, No. 2
Light Blue	Pale Chrome	Vermilion
Green Verditer	Roman Ochre	Crimson

Water-colors.—Those made by Winsor & Newton should be given the choice if one is able to pay for them. This expense, however, should not be considered wherever a true and permanent color is desired.

For general use in the laboratory, the Murillo Color-box, in either the 65 cent or 75 cent size, will give excellent results, and presents the advantage of containing, aside from the pigments, a number of brushes and a mixing-tray. The following list of colors can be had in the 75 cent box:

Vermilion	Prussian Blue	Burnt Sienna
Carmine	Indigo	Pale Chrome
Madder Brown	Ivory Black	Gamboge
Light Red	Emerald Green	Indian Yellow
Cobalt	Vandyke Brown	Yellow Ochre
Ultramarine		

The Colored Drawing Inks made by Higgins can be recommended; that is, if ready-mixed coloring fluids are to be used at all. They cost 25 cents a bottle and are made in the following tints:

Carmine	Blue	Brown
Scarlet	Yellow	Indigo
Vermilion	Green	Violet
Brick-red	Orange	

The directions for the use of Higgins' Inks, either the black or colored ones, are given in detail on the wrapper in which the bottle is packed.

Since some of the materials just described are not absolutely necessary for drawing of microscopical preparations, a summary of essential articles is given

below, all of which should be in the beginner's possession before any work of this kind is undertaken:

Drawing-board; half-dozen thumb-tacks; at least a half-dozen sheets of some good drawing-paper; three pencils, of which one must be hard, the other medium and the third soft; 1 pencil-eraser; half-dozen of Gillott's drawing pens, 3 of No. 290 and 3 of No. 659; 1 bottle of Higgins' black drawing ink; a set of colored crayons or water-colors; a good ruler, graded in millimeters; scissors for cutting paper; and a few blenders. Other articles may be obtained as occasion demands.

METHODS OF DRAWING.

DRAWING IN BLACK AND WHITE.

The drawing of microscopical preparations falls primarily into two classes: one done in black and white alone and the other in color. The first variety is by far the easier to execute and is more usually employed by microscopical

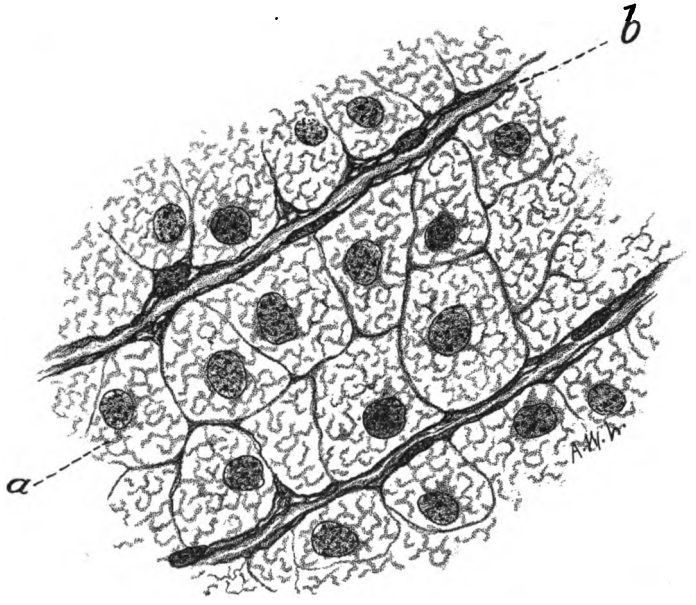

FIG. 5.—Detail from the zona fasciculata of suprarenal gland, human, and magnified about 750 diameters. An example of line and dot drawing, and also illustrating what can be done with No. 27 Ross Board.

workers. For these reasons the use of colors will be described later and in far less corresponding detail.

Four methods of distributing the pigment may be used, viz., in dots, in lines, in masses, and by combining two or all of these.

Dots are placed at will or according to the surface of the material drawn upon. If a pencil be drawn over any rough surface, the graphite will cling to

only the projecting portions, the spaces between remaining unaffected. In view of this, a vast variety of drawing-papers is manufactured, the surfaces of which consist of more or less minute elevations and depressions. Of all these, as remarked in the description of materials, only one finds extensive use in microscopical illustration, the Ross stipple board.

Fig. 5 shows a drawing made on No. 27, Ross stipple board, and illustrates

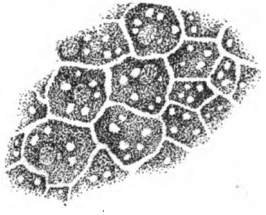

FIG. 6.—Cells from the pigmented stratum of the retina of rabbit, enlarged about 300 times; given to illustrate true dot-drawing upon a smooth paper.

how dots may be made according to the nature of the surface drawn upon. At *a* and *b*, this point is clearly pictured. Then again, dots may be made entirely according to the will of the operator; that is, each dot is made by a raising and a touching of the drawing tool to the surface. A drawing so executed is represented by Fig. 6.

While this latter figure does show a subject in which pure dot-drawing is not only permissible but imperative, it is not a very usual necessity throughout

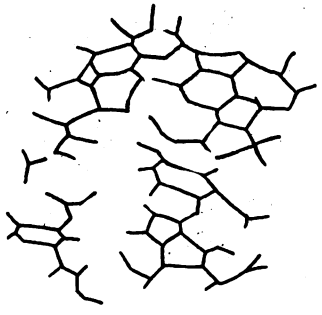

FIG. 7.—Detail from precipitation preparation of biliary capillaries, liver of rabbit, enlarged about 300 times. An illustration of pure line-drawing.

courses of laboratory work. Only when dots of a marked lack of uniformity of distribution are required is it necessary to place them in this way.

Lines are always drawn according to the will of the worker. They are likewise seldom the sole method of expressing a microscopical subject, although quite without a substitute in parts of all ordinary drawings. Fig. 7, however, illnstrates a case where pure line-drawing is the only way to tell the story.

Masses are done either by the use of a fluid pigment and a brush, by going over the surface carefully with a pencil until it is completely covered with graphite or by spreading evenly some powdered pigment with a tightly rolled blender of paper or leather. In certain instances, very true pictures may be made by the exclusive use of mass, as illustrated by Figure 8; but, it is more often a feature rather than the whole of a drawing.

Combination of two or all the preceeding styles is the commonest way of drawing microscopical preparations, and that each process may be understood, a drawing will be described which consists of dots, lines and masses in proportion to the demands of the selected subject.

A very important preliminary to starting a drawing of this kind is to carefully consider the arrangement of its larger elements. Never place an ample blood-vessel, a sharply circumscribed group of cells, lobes of a glandular organ,

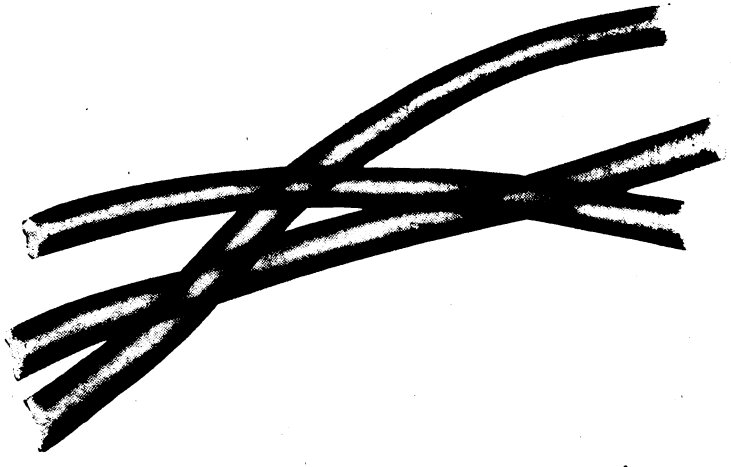

FIG. 8.—Silk fibers, enlarged about 300 times. An illustration of pure mass-drawing.

or, in short, any conspicuous feature exactly in the center of the drawing-field. A certain stiffness results if this is done and every means should be employed to avoid a mechanical effect untrue of the preparation.

It happens now and then, too, that one has come to an advanced stage in the drawing before it is observed that some imperfection of the drawing-paper occupies exactly the space that should be filled by a feature of the drawing. Therefore, only the best paper should be used, and even this must be examined closely to avoid the appearance in the illustration of specks, stains, breaks in the surface and other defects.

Again, it is very essential that the field to be drawn be carefully chosen. Foreign particles or artifacts produced by the treatment in preparing the specimen may be present, or, it may happen, that certain gross features of the preparation being drawn bear a close resemblance to some familiar or grotesque

form in macroscopic nature. Rudimentary faces, domestic animals, insects, etc., are occasionally simulated with such out-spoken likeness, that they appear more noticeably in the finished drawing than the histological details it was desired to present. Accordingly, that portion of the preparation where this unfortunate feature appears should be discarded for another. If this is not allowable, the preparation should be rotated this way and that until this similarity is least evident. An instance is recalled where a professional artist had failed to examine a preparation of digested tissue in regard to this point, and it was not until his drawing had been published that he remarked the all-to-life-like resemblance to a well-fed flea of a somewhat centrally-placed mass of connective tissue. By turning the preparation, from which this drawing was made, about 180 degrees, this unwelcome likeness dwindled to its faintest. It would have been less noticeable as well had it not occupied a central position.

Magnification.—The purpose of a drawing, naturally, may be as varied as investigation itself. But, in another direction, the purpose of an illustration is quite limited; that is, to show the structures in correct proportions under the magnification to which the preparation examined has been subjected. If the object is enlarged, say 30 diameters, its larger features will be much more prominent to the student than the minute ones. If the same object be magnified 1500 times, however, its larger features will vanish and those of minute dimensions now play the chief part. The matter of relative size of parts has always to be borne in mind, and frequently a sense of proportion has to be cultivated. Between these two extremes of magnification, there is a stage where neither minute nor gross relations so greatly dominate the picture, and, fortunately, it is with this intermediate level that beginners have most to do. Size distortion must always be avoided. How unnatural it would be to draw outlines at a magnification of 200 and to fill in with details of twice that enlargement A certain modification of this last statement is occasionally allowable, as explained under the remarks on Detail.

The Outline.—There is no time in the history of a drawing when more thought and observation is necessary than at the placing of its outline. As far as appearances go, the outline plays but a secondary part in the completed picture, yet, it must not be forgotten, that it is in fact the frame-work upon which the whole structure rests. A good outline may redeem bad finish, but no amount of excellent finish can save a picture that has been incorrectly outlined.

The pencil used for this purpose should be well sharpened and of medium grade. HB will be found quite satisfactory. Many beginners make the error of using a very hard pencil for outline work. It is quite true that an unsteady hand can make finer lines with a hard than a soft pencil, but an unsteady hand is apt to be a heavy one as well, and at the same time that a line is being made the surface of the paper is being scratched, a fact not realized until erasure is attempted. And erasure will be frequently resorted to during the first efforts, because of false drawing. Therefore, every precaution should be taken to avoid making lines that cannot be erased with ease. Unless great pressure be

put upon an HB pencil, its use will be unattended with deep indentation of the paper or a scratching of it.

Before beginning the outline proper in making Figure 9, the *limits of the drawing-field* were placed, *a*, indicating the lower one. These limits can be placed according to the choice of the operator or the demands of the subject; but the quadrangular style, as here pictured, enjoys at present considerable favor. Frequently drawings of irregular boundary are made (Fig. 6) and then no limiting lines are necessary.

After placing the limits of the drawing, a careful attempt should be made to locate some tissue-element that passes approximately through either the

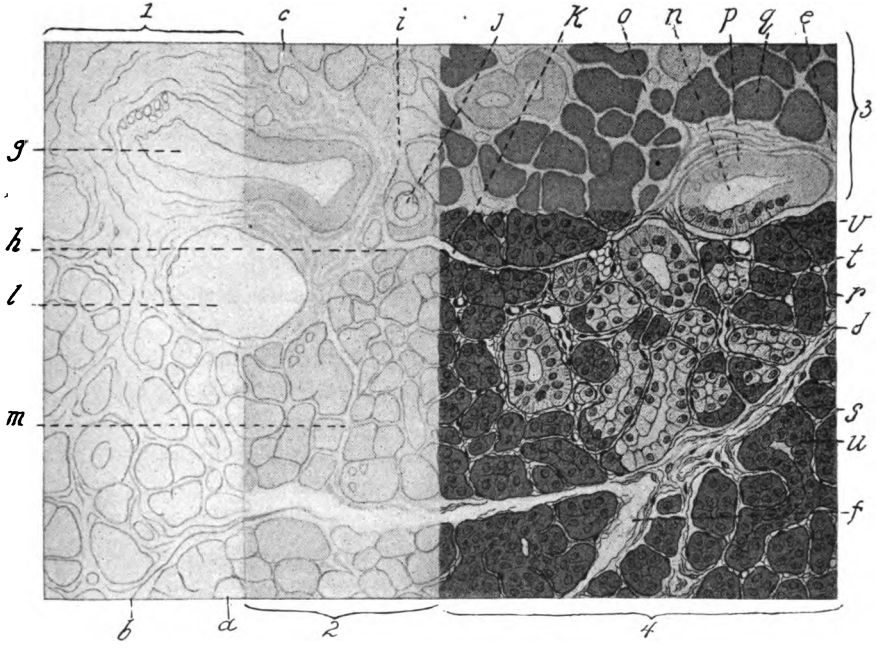

FIG. 9.—Taken from the human submaxillary gland, enlarged 125 diameters. Given to show the different steps in making a microscopical illustration.

length or breadth of the area. If such cannot be found, then let some prominent feature be sketched. Since all subsequent outlines are placed with constant reference to these first features, they may well be termed *trunk-lines*, and as such will be spoken of in the following description. The trunk-line of Fig. 9 was taken from a roughly, linear mass of connective tissue. Irregularly Y-shaped, its stem reaches the lower limit of the picture at *b*, and its limbs are cut by the upper and right margins at *c* and *d* respectively. Since this Y-shaped mass of connective tissue was drawn in only as a single line, naturally no attempt was made to indicate its exact width or special characteristics; merely its posi-

tion in the selected field and its curves and angles were noted. The object of the trunk-line is to give the observer some fixed point, from which he can proceed to place the more definite outlines of the subjective features in the same relative size and position which they possess in the preparation. This is a very important step, and while an expert might ignore it entirely, the beginner will find such a line of great service toward giving the drawing its proper proportion.

From this Y-shaped connective-tissue mass, it was observed that smaller branches further subdivided the subjective features, and these were hence sketched in as elaborations of the trunk-line. Such may be seen at *e* and *f*. These lines were then utilized in the following manner to outline the remaining tissue elements.

At *g*, the outlines of a somewhat tangentially-cut duct can be seen, and in the preparation its right limit ran about parallel with that portion of the left limb of the Y-shaped connective-tissue mass included between the point *h*, where an interlobular septum joined it, and the point *i*, where this limb bent sharply toward the left. Further, this right limit of the duct *g*, was observed to be as far to the left of the extreme right limit of the connective-tissue mass as the lumen of the blood-vessel *j*, was wide.

Looking at the long axis of the section of the duct *g*, it was seen that it inclined upward at about the same angle as that portion of the interlobular septum which is indicated at *k*. This latter observation determined the direction of the duct's long axis but not its length. To establish this, attention was directed to the right-hand limit of the duct, which had already been sketched. The length of this limiting line was picked up, as it were, in the eye and then laid upon the long axis of the duct in much the same manner as a carpenter uses his rule. By means of this comparison it was determined that the long axis of the duct was about three times as long as the line used as a measuring unit, and a point was put down at that distance to mark off the extreme left limit of the duct. This left-hand limit was sharply bent at about its middle, and by drawing an imaginary line between the ends of this bend, the left limit was found to be about the same length as the right limit. Near its middle portion the duct was constricted to about three-fourths the breadth of the right-hand limit. With the dimensions recorded, the duct was then outlined, giving particular attention to its inner and outer contour as formed by cellular limits. The next feature sketched in was the large blood-vessel, *l*. The same methods of placing it in proper proportion were employed as those described in sketching the duct, *g*. Thus, three features were gained as measuring units: the Y-shaped connective-tissue mass, the tangentially-cut duct, and the large blood-vessel. Constantly using these as guides, the surrounding tissue elements were outlined, each being added on by continual intercomparison, and, in this manner, were placed, if not in unerring size-ratio, at least, in fair proportionate likeness to the actual specimen.

It has taken some space to describe this process of outlining in proper proportion, but as soon as the beginner has grasped the idea, he will use it with

considerable speed and exactness; in fact, it shortly becomes an almost unconscious procedure.

The Ground Color.—By this term is meant an even mass of faint color distributed over the whole drawing except where the transmitted light from the reflecting mirror of the microscope passes unimpeded through actual spaces in the tissue. If those spaces are small, the ground color may be carried over them without hesitancy; but if they are conspicuous, as the interlobular area, *m*, in Fig. 9, the ground color must be omitted. The ground color, as it was laid in Fig. 9, is left unchanged in that portion of the illustration included within the bracket 2. Bracket 1, shows a section representing the outline alone. This ground color is to the various shades of the picture what the trunk-line is to the sketching in of the tissue elements. It holds these shades together and is used as the unit of their intensities. This point will be more fully explained at the stage of Differential Color.

There are three methods in common use in laying the ground color: (1) by lightly drawing a pencil back and forth over the paper until an even coat of graphite is obtained; (2) by charging a tortillion with some powdered pigment, usually graphite, and carrying it over the paper with a light touch until a uniform mass of color is effected; and (3) by brushing on some fluid pigment, as a rule, an aqueous solution of black water-color, process black or drawing ink.

1. If the pencil be chosen with which to lay the ground color, it should be neither too hard nor too soft. A very hard pencil requires considerable pressure, while a soft one must be handled with extreme delicacy to obtain an even mass of pigment. HB, Koh-I-Noor, or Faber will be found a satisfactory grade of pencil at this stage of the drawing. Before commencing the procedure, however, the pencil's point must be dragged back and forth over some rough scratch-paper, in order to blunt it, as a sharp point is apt to give a lot of fine lines, rather than an even mass of color, as well as delay the process. Should lines occur, in spite of the mentioned precaution, they may be blended into a fairly homogeneous mass by rubbing over them with a clean tortillion; that is, if they have not been put in too heavily. The beginner will probably find the pencil, with subsequent use of the tortillion, by far the most satisfactory method of laying the ground color, since with them there is less danger of its overlapping its proper boundaries. The charged tortillion and the brush can be used with good results only after considerable familiarity with their particular characteristics has been gained.

2. If powdered graphite be the pigment used to lay the ground color, the employment of the tortillion is necessary. The graphite is prepared in the following manner: a file of fine tooth is held at an angle over a small receptacle or piece of paper, and a pencil of moderate softness, HB, for example, is dragged up and down upon the file. Into these filings the tortillion is now rubbed, is then gently tapped with the finger to remove any superfluous pigment clinging to it, and is finally gently rubbed over the scratch-paper, turning it meanwhile, in order to distribute the graphite evenly upon its point. The tortillion, thus

charged, is now to be used on the drawing exactly as a pencil. If the ground color shows streaks, it may be converted into a uniform mass by rubbing over it with an uncharged tortillion. It will be found that the tortillion's point, after some use, has become worn off and quite flexible, an occurrence which rules out its further employment in the fine work. For drawings of small area and requiring a light shade of the ground color, the necessary graphite may be made available by simply rubbing the point of the HB pencil, flat-wise, over an area of an extra piece of drawing board until the surface is densely black (thus obviating the need of a file) and applying the tortillion to this surface.

3. With a brush and fluid pigment the best ground color can be laid. The brush, however, is such a stranger in the hands of most, that the beginner seldom accomplishes good work with it. Practice will, nevertheless, enable one

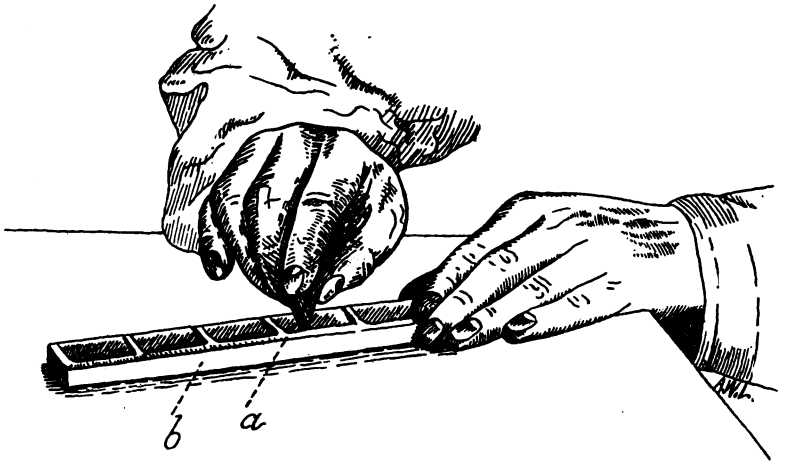

FIG. 10.—Illustrating the method of preparing fluid pigments from the solid block.

to pursue the method with increasingly better results, and because of its greater possibilities, its use is strongly recommended. As pigment, either a solid or fluid variety may be employed, and of these Winsor & Newton's Ivory Black and Process Black may serve as respective examples. A solution of Ivory Black, from the solid cake, is prepared as follows, illustrated by Fig. 10:

The color-block, *a*, is firmly held between the thumb and first two fingers and then rubbed vigorously in a few drops of water placed in one of the compartments of the mixing-dish *b*. Gradually the water will take up the color until it assumes a deep black tone. Then the trituration of the color should be stopped and clear water added to the fluid in the mixing-dish until it is about twice as dark as the tint desired for the ground color. The brush is charged with this fluid simply by immersing it in the same and allowing it to draw as much of the pigmented water as it will. Here the beginner is apt to make a mistake by freeing the brush of some of its charge until its point is finely tapered.

This should not be done, as, in that case, enough fluid does not remain in the brush to permit the laying of an even mass of color. If the brush has been charged properly, an excess of fluid will be left in its trail over the drawing, and, since the color has been prepared twice the intensity of the tone desired, it will seem much too dark. No anxiety should be felt at this, however, as the following step corrects the apparent mistake: The brush is sharply shaken over a hopper until relieved of the greater part of its charge of color, which is evidenced by the

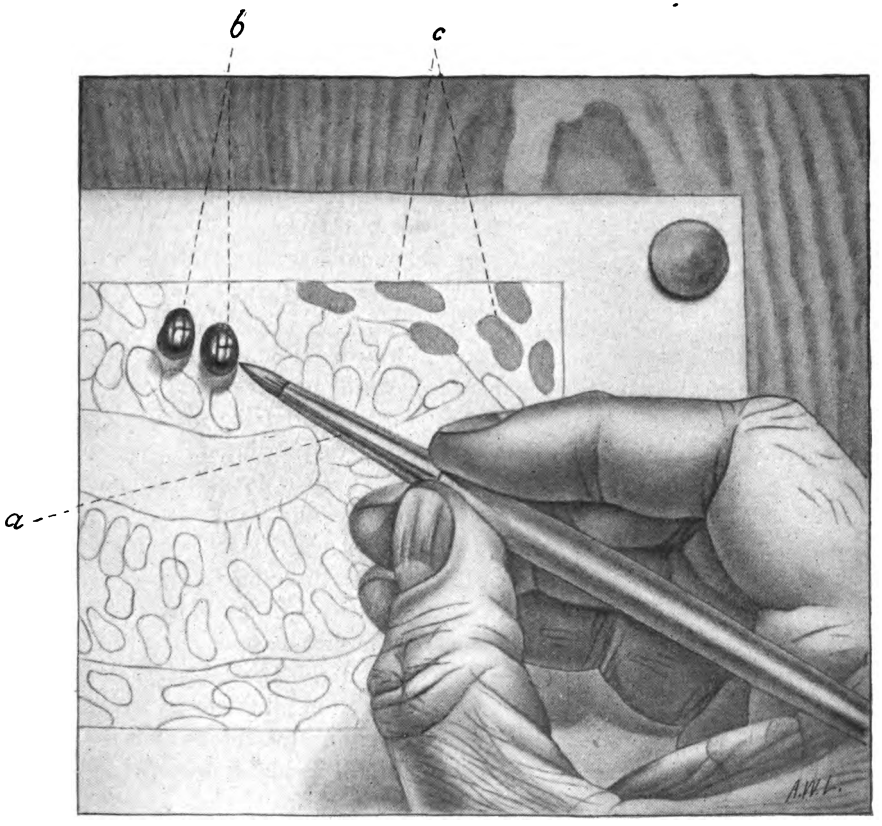

FIG. 11.—Illustrating the method of removing the excess of color in the process of using fluid pigment for placing the ground-color or differential color of a drawing.

reappearance of the tapered point. The exhausted brush is now to be carried over the area on the drawing where the excess of fluid presents, and, just as the hairs absorbed the pigmented water from the mixing-tray, they now do the same from the drawing. Since only a portion of the suspended pigment has had time to settle to the surface of the drawing, this process of removing the excess of coloring fluid will leave a much lighter tint than one would expect to follow the use of a color so dark. Accordingly, it must be borne in mind that the pigment should always be prepared considerably darker than the tint

desired for the ground color. To determine just how dark this pigment should be during the mixing, a few tests on scratch-paper similar to the paper drawn upon will be necessary for the beginner. Fig. 11 gives an idea of how the surplus of coloring fluid is removed from the drawing. The brush, *a*, has been exhausted, as is shown by its finely tapered point, and is about to be plunged into one of the drops of excess coloring fluid, *b*. *C*, represents areas that have already been relieved of their surplus color. The object in following this procedure, instead of merely painting over the desired surfaces with a partially exhausted brush, as the beginner is prone to do, is to obtain an even distribution of the ground color, and it must be realized that this is attained by the settling of the suspended particles of pigment upon the surface of the paper from the excess of fluid above. Therefore, care should be taken that the excess remains equal lengths of time upon the paper. If the areas to be colored are small and individual, the color may be placed upon three or four at a time and the surplus removed in the order in which it was put down.

Proceeding as directed above, the ground color was then laid on in Fig. 9, a portion of which, as remarked, is still to be seen in that part of the picture included within the bracket 2.

Differential Color.—This stage of the drawing is one generally attended with as much difficulty for the beginner as even the proper placing of outlines, and it is here that most drawings depart furthest from the specimen under observation. By differential color is meant that varying intensity of shading, which serves to show the different tints in stained preparations on the one hand, or, on the other, the relative opacity of structures in unstained ones. To illustrate this point, attention is directed to that portion of Fig. 9 included within the bracket 3. There it may be seen that the only uncolored areas are where there is no tissue to obstruct the rays of transmitted light, as in the lumen of the duct, *n*. Proceeding from light to dark, the first mass of color to attract attention is the remnants of the ground color, as at *o*. The next darkest shade is to be found in the ducts, as at *p;* and the darkest of all the tones seen in the field, included by the bracket 3, is that representing the serous alveoli, as at *q*. The darkest differential shade in the whole drawing, however, is shown in the connective-tissue fibrils, the connective-tissue nuclei, protoplasmic granules and reticulated basement membranes, all of which are included within bracket 4. But since this dead black color belongs to the stage of Detail, it will be postponed until that subject is discussed.

Before laying the differential color on the drawing, according as the tissue is stained or unstained, the respective tints or degrees of opacity in the preparation must be closely scrutinized, and the point settled how to express them in black or in some degree of its intensity. In the preparation from which Fig. 9 was drawn, the following colors were observed: The lightest color was found to be a very faint, cloudy blue presented by the mucous cells, *r*. The next lightest was a brick-red in the connective tissue and ducts, as at *s* and *t*, respectively. Following that, the serous alveoli, as at *u*, showed the next

FIG. 12.—Scheme showing the relations of the principal colors to each other. Inner circle, Primary Colors; middle circle, Secondary Colors; outer circle, Tertiary Colors.

degree of shading, a reddish purple, while some of the connective-tissue nuclei gave the darkest tone of all in the way of a heavy hematoxylin stain, bluish purple, *v.*

Though the subject of color is discussed later, it is right here very advisable to devote some time to the science of color, as its elementary principles will help the beginner to make better drawings in black and white.

Fig. 12 shows a number of colors arranged concentrically in three circles. The three colors included in the central circle are yellow, red and blue, called primary colors, since they cannot be obtained by mixing other tints. The next circle includes the secondary colors; that is, those obtained by mixing the two adjacent primaries, thus: blue mixed with yellow gives green; yellow mixed with red gives orange, and red mixed with blue gives purple. The outermost

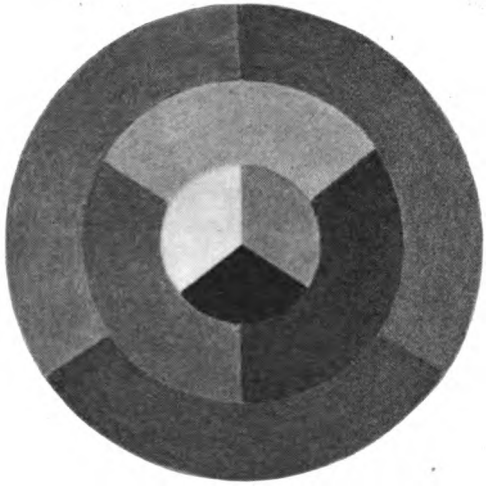

FIG. 13.—Companion scheme to Fig. 12. The different shades of black comprising the three circles are intended to represent in terms of black and white, the shade-value of the respective colors presented in Fig. 12.

circle contains the tertiary colors, which are obtained by mixing the two adjacent secondaries, thus: green with orange gives olive, orange with purple gives brown, and purple with green gives gray.

Directing attention to the primary colors, it is easily evident that yellow gives the impression of containing more light in it than either red or blue, while blue is the darkest of the three. For this reason yellow is spoken of as light, blue as shadow, while red is taken as the symbol of color. Among the secondary colors, orange is the lightest, because it is produced by mixing the two lightest primaries, yellow and red. Green is not so light as orange, due to its component of blue. Purple is the darkest secondary color, as it has no yellow integer. Of the tertiary colors, olive is the lightest, since it contains two parts of yellow coming from the colors used to make it, orange and green, and only one part of blue, derived from the green. Brown is darker than olive,

since by analyzing its component colors, orange and purple, it is seen that one part of yellow has been supplanted by an integer of red, purple containing no yellow. Gray is the darkest of the tertiary series, since inspection of its integral colors, green and purple, will show that it contains two parts of blue, which represents more shadow than exists in either olive or brown.

Now, comparing the colors in the three rings, one with the other, in regard to their relative lightness and darkness, it is readily seen that yellow is the lightest, orange the next and olive the next. Turning to the other extreme, blue is found to be the darkest color, purple follows second, while gray is the third in shadow intensity. By analysis of these colors, according to the number of yellow units they contain, on the one hand, and the number of blue units, on the other, it can easily be explained why they bear to each other this respective relationship of light and shadow.

This discussion of color science has been given to show the means by which color proper, or color values, may be translated into black or some degree of its intensity. It is obvious, of course, that this cannot be done so perfectly that mere inspection of these varying degrees of black will reveal exactly the colors they were designed to represent, nor is this at all necessary for our purpose. It is, however, quite possible to make a drawing in black and various degrees of its intensity in which the color differentiations of the tissue elements will be nearly as sharply distinguished as in the preparation under the microscope. To illustrate this, attention is called to Fig. 13. Here again are three concentric rings. In the inner circle the lightest area is pure white and represents yellow; the darkest area stands for blue, while the remaining one represents red. In the second circle, the lightest part corresponds to orange, the darkest represents purple, and the remaining area stands for green. In the outer circle, the lightest area represents olive, the darkest one, gray, while the remaining area represents brown. As already remarked, a critical examination of these varying degrees of black would surely fail to distinguish the exact color each is intended to represent; but by comparing them with the colors in Fig. 12, which occupy the same relative positions, a marked similarity will be found between the integers of both schemes; that is, as far as light and shadow are concerned. For example, the lightest color in Fig. 12 is yellow, while the lightest area in Fig. 13 occupies the same relative position. Further, blue is the darkest color in Fig. 12, and in the same relative position is to be found the darkest area of Fig. 13. Such relations will be found to tally throughout the balance of both figures.

For the practical application of these relationships, suppose a preparation which has been subjected to the action of two stains, blue and yellow, is to be represented in a black and white drawing. Reference to Figs. 12 and 13 shows that blue is many times darker than yellow, so the two differently stained tissues would be accordingly differentiated in the drawing by the use of a strong degree of black for the blue and a much weaker degree for the yellow.

This, however, may be done only in instances where the blue and the yellow are in sufficient quantity to be considered approximately pure.

A condition found quite commonly in stained preparations, unfortunately, brings a difficulty into the matter not to be solved by the use of constant rules. This difficulty is the one of color dilution, and must be met by the student's individual powers of interpretation. True blue is darker than true red; but a blue that has been diluted to an extreme degree with white light (decolorized or not taken by the tissue), could not properly be represented by a stronger intensity of black than the one used to represent a true red. To illustrate this, a common enough landscape suffices. Imagine the pale blue sky of a day in early spring, and, contrasted with it, the red roof of a nearby house. If a pencil sketch were to be made of this combination, how unnatural it would look if the roof were drawn in lighter than the sky! And diluted colors—that is, very pale ones —will probably be found more often in stained tissue than will colors of full intensity. To satisfactorily represent these in degrees of black, the student is thrown entirely upon his own resources. Nevertheless, he will be better prepared to interpret the shade values of different tints if the foregoing elemental principles of color science have been carefully considered.

Turning back to the drawing represented in Fig. 9, which has been described up to the stage of Differential Color, the various tints which were seen in the tissue under the microscope will be enumerated again. The lightest color was a very much diluted blue, shown by the mucous cells; the next lightest, a brick-red in the connective tissue and ducts; the next, a reddish-purple in the cells of the serous alveoli, and the darkest color of all, a bluish-purple, was found in some of the connective-tissue nuclei. With the exception of the latter, these colors were represented in the drawing by various degrees of black, as seen in that portion of Fig. 9 included within bracket 3. It should be remembered, in placing these shades, that the intensity of the ground color must be constantly kept in mind, as it is the unit of shading in the picture. It represents, as said before, the lightest structure in the preparation, and, since the mucous cells presented the lightest color to be found in the preparation from which Fig. 9 was drawn, the ground color on these was therefore left quite unchanged and all other degrees of black were laid to properly contrast with this tone. Thus the ground color is to the differential color what the trunk-lines are to the placing of the outline.

Just as in putting on the ground color, the differential color may be laid either with pencil, pencil and tortillion, with the latter alone charged with powdered graphite, or, with a brush and fluid pigment. The latter was the method used in drawing Fig. 9. The same advantages which the pencil and tortillion offer the beginner in placing the ground color also hold good when laying the differential color. The charged tortillion and brush with fluid pigment are by no means so easily handled. Both of them are apt to overlap the tissue outlines, especially if the areas thus treated are of small extent.

The Stage of Detail.—This is the period in the history of a drawing when

the final characteristics of the tissue elements, the details of structure, are added. These are shown in Fig. 9 at that portion included by bracket 4, and are seen to consist of individual connective-tissue fibrils, cell boundaries, protoplasmic granules and nuclei.

Concerning what details should be pictured and what left out depends entirely upon the purpose of the drawing, and thus the amount and kind of detail obviously will differ with the special object of the illustration. Again, granting that but one drawing is to be made of a certain preparation, as in Fig. 9, and that the object of the drawing is to give a general idea of the structure of that preparation, it is then permissible to slightly intensify the appearance of some details which are only imperfectly seen at the given magnification. For instance, the striations found in the duct-epithelium of Fig. 9, at *v*, could not be seen distinctly at the enlargement of 125 diameters, but their existence and character should be shown in the general drawing. Raising the magnification, however, to 500, these markings could be definitely made out, and they were put in as seen to be arranged under this magnification. Also, many of the connective-tissue fibrils were by no means as sharply evident as represented in the drawing; but, as this one drawing is to express a fairly comprehensive idea of the tissue constituents of the preparation, and since increased magnification made the fibrils quite clear, no hesitancy was felt in making the fibrils more apparent than they were at an enlargement of 125 diameters.

It will be noted that some of the cell boundaries, such as those found in the serous alveoli, are exceedingly faint. This detail was accomplished not so much by the fineness of the line as by a dilution of the drawing fluid. This may be resorted to successfully in many instances where one wishes to represent features but faintly seen in the preparation. Had it been especially essential to the purpose of the drawing to exagerate these cell boundaries, such would have been done as in the two instances above mentioned; but in this case mere indication of them sufficed.

In *diluting* any drawing fluid, something should be added that it may have sufficient consistency to prevent the pigment granules gravitating immediately to the pen's point. The following manner of dilution will be found efficient: Having put a few drops of ink or triturated water-color in the mixing-dish, water is added until the desired intensity has been reached, and then, with a brush whose point has been dipped in Le Page's glue or a 10 per cent. aqueous solution of gum arabic, the whole is thoroughly mixed. This diluted fluid is put on the pen with the charged brush. Concerning the choice of pens for placing details, reference may be made to the pages dealing with drawing materials.

Lettering.—The details placed, the drawing is now finished, aside from putting in the various "leaders," which refer to structures discussed, and lettering them. These leaders may be made either as solid lines or broken ones, the selection being left to the operator with but this one condition: neither should be made so thick that it obscures those parts of the drawing through which it passes. Broken lines give neater effects and obscure less, but they

are more difficult to make uniformly. There is a great number of styles used in lettering, and here again but one condition is imposed—legibility. One form of letter is, however, particularly popular and is very easily read, namely, the so-called italic. The characters of this style of letter may be seen in the following alphabet and numerals:

Aa, Bb, Cc, Dd, Ee, Ff, Gg, Hh, Ii, Jj, Kk, Ll, Mm, Nn, Oo, Pp, Qq, Rr, Ss, Tt, Uu, Vv, Ww, Xx, Yy, Zz, 1, 2, 3, 4, 5, 6, 7, 8, 9, 0.

The placing of characters within the drawing on the structures described is to be condemned. This not only obscures the detail but frequently occasions considerable annoyance, as letters or numbers so placed are difficult to find, especially if the drawing is of any size and rich in detail. Fig. 9 gives an example of lettering, and while the leaders are placed irregularly here, they

FIG. 14.—Illustrating use of apparatus and position assumed in "fixing" a drawing.

may often be placed radially or even run parallel to each other. At times, however, parallel leaders give an effect of stiffness to the drawing.

Fixing.—The materials used in this process have already been described. The process is necessary only in those instances where pigments have been used that have a tendency to rub.

Fig. 14 shows the position assumed by the operator when fixing a drawing. The drawing-board, *a*, upon which the illustration, *b*, has been fastened with thumb-tacks, is held in the right hand at arm's length from the body. The left hand supports the bottle of fixing solution, *c*, in which the cylindrical tube, *d*, of the atomizer is immersed, while the flattened tube, *e*, is held firmly between the lips. Upon strong blowing through the mouth-tube a jet of air streams swiftly across the upper end of the tube resting in the solution, thereby causing a reduction of pressure within it, which, in turn, causes the fluid to rise. When this column of fluid comes in contact with the jet of air from the mouth-tube, it is divided into a fine spray which deposits itself evenly over the drawing. If the drawing be held close to the bottle, where the spray is more dense, it will

be literally bathed rather than lightly sprinkled with the fluid. This is a condition to be avoided, since a large quantity of fixing solution takes some time to dry, leaving, as well, an eye-trying gloss upon the picture. Again, if considerable graphite be present, a surplus of the solution may carry it down along the picture giving it a streaky appearance upon drying. To obtain the best spray, the mouth-tube should be held at nearly a right angle to the immersed tube. A marked obtuse angle will result in directing the jet of air into, instead of across the tube, or an acute angle, so far away from its upper end, that the fluid will not rise. After use, the atomizer should be shaken sharply to free it of the fixing solution still contained in the cylindrical tube, for, if this were allowed to remain, the evaporation of the fluid would leave a plug of resin in the tube's chamber, an obstacle that often causes considerable vexation before its removal is accomplished.

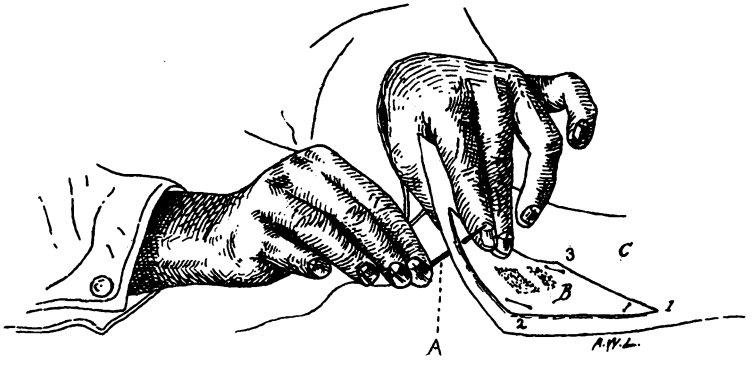

FIG. 15.—Showing a method and a form of stitch to be used in mounting drawings by sewing.

Mounting.—In common use there are three methods of mounting the drawings in laboratory papers: pasting, sewing, and insertion in slits. Each has its particular merits, but the last method is least burdened with disadvantages.

If pasting is selected, only a fluid of rapid and effective adhesive qualities should be used, such as Le Page's liquid glue. If an inferior mucilage has been chosen, it will take some time to "set," and even then there is no guarantee that the drawing will hold to the mount. No attempt should be made to cover the whole back of the drawing with glue, as in this instance it will be found only too often that the drawing upon drying has acquired unsightly folds due to unequal contraction of the paper fibres. The better way is to sparingly brush the glue on the reverse side of the drawing only at the corners. Since the tendency of most workers in handling glue is to smear it in many places aside from where it is needed, and because of the manifest difficulty of removing a drawing so mounted, which occasionally must be done, the use of the glue is not warmly advocated.

Sewing is a clean and simple way of mounting a drawing and in many instances quite efficient. It presents, nevertheless, two unpleasant features: If the paper is much handled, in which sewn-on drawings are contained, the frequent bending of the pages leads to a sharp strain on the threads causing them to tear through. This is particularly common if the drawings are mounted on thin paper. Again, the removal of sewn-on drawings takes time and some manipulation. Fig. 15 gives an idea how to place the stitches. The right hand is in the act of pushing the needle, *A*, armed with stout thread, through the mount, *C*, and the drawing, *B*. When this has been done, the thread is

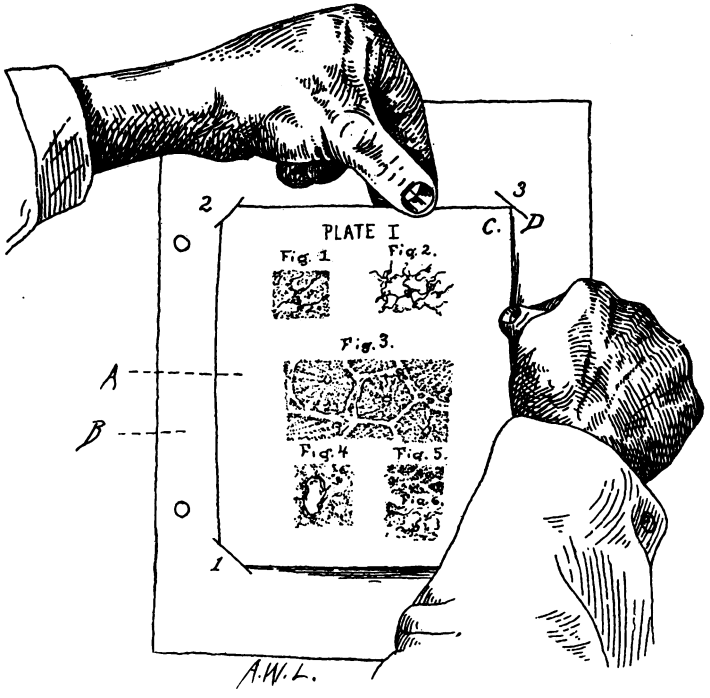

FIG. 16.—Illustrating the method of mounting drawings by the use of slits in the mounting page.

pulled through and the needle carried again through drawing and mount, forming the stitch shown. The thread is then securely knotted on the reverse side of the mount. At 1, 2 and 3, the stitches already placed show their diagonal relation to the corners of the drawing.

Fig. 16 gives a convenient and simpler method of mounting a drawing. It consists of slipping the corners into slits. The ease and neatness, with which this can be done, as well as the simplicity of removing the drawing at any time, strongly advocate its adoption. Its one weak point is that, with much handling, one or more corners may become disengaged and the drawing fall

from the mount. This can be avoided largely by making the slits at such a position that considerable of the corners will slide into them. At 1 and 2, the corners of the drawing, *A*, have been inserted into the slits through the mounting paper, *B*, while at 3, the corner, *C*, is drawn back to a point where, by letting the drawing assume its natural shape, it will slip easily into the slit, *D*.

DRAWING IN COLOR.

To do justice to the use of colors in making illustrations of microscopical subjects, a great deal more space would be necessary than is here available. It is well realized that both the judgment and the art necessary for the advantageous use of color can only be attained by considerable training. They are often apparently inherent. In an ordinary class of students, relatively so few display any true skill in the handling of colors that it is advisable to discourage their general use.

However, some structures are classified according to their color-reaction when treated with certain stains; consequently drawings of them must be made in color. While in this, as in all things, practice will do so much for even the most ungifted, a wise rule for a class studying microscopic anatomy is, *not to attempt the use of colors except in those instances in which color differences or color differentiations are the especial and distinctive features of the structures under consideration.* For example, the stain or color reactions of the cytoplasmic granules of the white blood-corpuscles are distinctive characters of these corpuscles and form one of the bases upon which they are classified, and therefore, must be represented in colors; or, again, the color differentiation of the neuroglia is the chief character by which it may be distinguished from the white fibrous tissue with which it is mixed in the central nervous system and, in drawings involving both these tissues, the use of color is quite essential to the proper imitation of the features by which they are recognized. But in all ordinary cases, less time will be consumed and cleaner and more concise results will be obtained with drawings in black and white.

In the use of colors, the general student must choose one or the other of the two simpler methods of their application, namely, the superimposing of crayons or of water-colors upon the outlines and certain details of structure already drawn in with pen and ink. Of the two methods, the use of crayons is the easier. This will be described first, followed by directions for the use of water-colors, and a drawing, given in steps, which will serve to illustrate both methods.

Crayons.—First, place the outlines and such details as may be represented by lines, with pen and ink, then give the required color to the different structures by simply rubbing the proper crayons over them. The combination of pen and ink with crayons will give much better results in the hands of most than if the drawings be made exclusively with crayons. The different crayons have already been discussed sufficiently in the description of materials. When

filling in such small areas as nuclei, the crayon should have a sharp point. Larger surfaces, however, are more evenly covered when the point is blunt.

One principle in laying in the different tints with crayons is exactly the same as that described in the paragraphs on black and white drawing, namely, always work from light to dark tones, since, in the use of crayons, a dark tone, once placed, cannot be made much lighter by superimposing a lighter color, and it is likewise impossible to erase it all away. But, the lightest tint in colored drawings cannot always be carried over the whole drawing, as may be done in the use of black pigment, since its presence may often render the correct coloring of certain tissue elements an impossibility. For example, suppose a portion of a preparation takes a purple stain; then, an underlying tone of yellow would entirely preclude the placing of purple, since, as already shown, purple contains no yellow integer. If, however, as is the case with most preparations stained with hematoxylin and eosin or Van Gieson, the lightest color is yellow or yellowish-red, and runs all through the different tints of the preparation, then yellow should naturally be laid in one mass over the entire drawing. It would be least evident in the areas occupied by nuclei, for example, but could be found in all the elements of the preparation, and must, therefore, be in all the elements of the completed drawing, modified, of course, by the superimposing of the other colors required.

To obtain differential tints, a little analysis may be necessary. For example, suppose certain of the keratinous structures, as in sections of hairs or of thick skin, take a greenish tinge; then, this tinge may be accomplished by superimposing blue upon the existing yellow, the depth of the green depending upon the mixture used. Yellow-brown tints may be made by superimposing Roman ocre upon the yellow ground color. The tones of red, blue or purple of the nuclei may be attained likewise by the proper mixing in the proportions found necessary.

In placing the differential colors, it will be found that the superimposing of colors to get the required tints frequently gives truer and more pleasing results than are to be obtained by the use, often practiced, of a single crayon for each color. This is so chiefly because the prepared pigments seldom truly imitate the colors of the stains used.

Two important precautions are to be observed in the use of colored crayons which, as described under materials, consist largely of wax; namely, *crayon should not be blended with the tortillion and should never be fixed.* The wax does not permit of blending but will clump under the pressure and rubbing, and wax is soluble in the fluid portion, the alcohol, of the fixing solution, a property that will occasion a total ruin of the drawing if the fixing agent is used.

Water Colors.—The various articles used in this style of drawing are described in the discussion of materials. Water colors may be applied with a brush exactly as crayons, that is, they may be distributed over the tissue details which have previously been drawn in with water-proof ink. Or, again, water colors may be so dispensed with both brush and pen that the resulting

picture more closely resembles the tissues examined than one made by any other style of drawing. An example of this method of applying color is given in Fig. 17. This drawing was executed in much the manner as that practiced with the shades of black in Fig. 9, and the steps of procedure are much the same as required in the use of crayons. First the outline and linear details were placed, then the ground color; following that, the differential colors, and lastly, the finer structural details. Portions of the drawing involving these different stages are inclosed by the brackets 1, 2, 3, and 4 respectively.

The stage of outline here is in all respects quite the same as that described in making black and white drawings. The ground color, however, differs from the one laid in Fig. 9, in the fact that true colors were used instead of some degree of black. As previously cautioned, a careful inspection of the different tints in the preparation must be made before attempting to lay on the ground color. This inspection of the preparation, from which Fig. 17 was drawn, gave the following findings: The lightest tint was presented by the tangentially cut hairs, within their follicles, a pale reddish yellow as seen at *a*. The next lightest tint, a much diluted red, was found in the sebum, *b*, the partially disintegrated sebaceous cells, *c*, and in the sebaceous cells still intact, *d*. Following this light red, the next lightest tint, a pale purple, could be seen underlying the nuclei of the hair-sheath, *e*, and the sebaceous duct, *f*. A rather strong yellow-red came next in order, as presented by the connective tissue at *g*. The details of the latter and the outlines of the sebaceous cells, *h* and *i*, respectively, gave the next darkest tint in the way of a brick-red, while the darkest color of all could be seen as a strong purple in the various nuclei, as at *j*.

Accordingly, the three primary colors were found, mixed, it is true, here and there to give some of the secondary colors. As was stated in the discussion on the use of crayons, it is always advisable to work from light to dark, and this principle should be observed in the use of water colors. Therefore, the lightest integers in the different tints of the preparation constitute the ground color, and may be seen in Fig. 17 as yellow in the connective tissue and tangentially cut hair, *k* and *l*, respectively, and as red in the hair-sheath, *m*.

The stage of differential color has been left unchanged in the area included by bracket 3, and shows how the color of the connective tissue bundles, *g*, was accomplished by superimposing red on the existing yellow, and how the delicate purple, *e*, underlying the nuclei of the hair-sheath, was effected by washing blue over the red already placed in the ground color.

This method of getting desired tints by superimposing water colors will be found as satisfactory as in the use of crayons, and for the very same reason given under that head. It is, however, now and then necessary, and quite often practiced, to first attain the desired tints by bringing the colors directly together in the mixing-dish and subsequently applying them with the brush to the drawing. This was done to represent the body color of the nuclei in Fig. 17. Furthermore, both the ground and differential colors should be brushed on in excess, and then this surplus color is to be removed by use of the

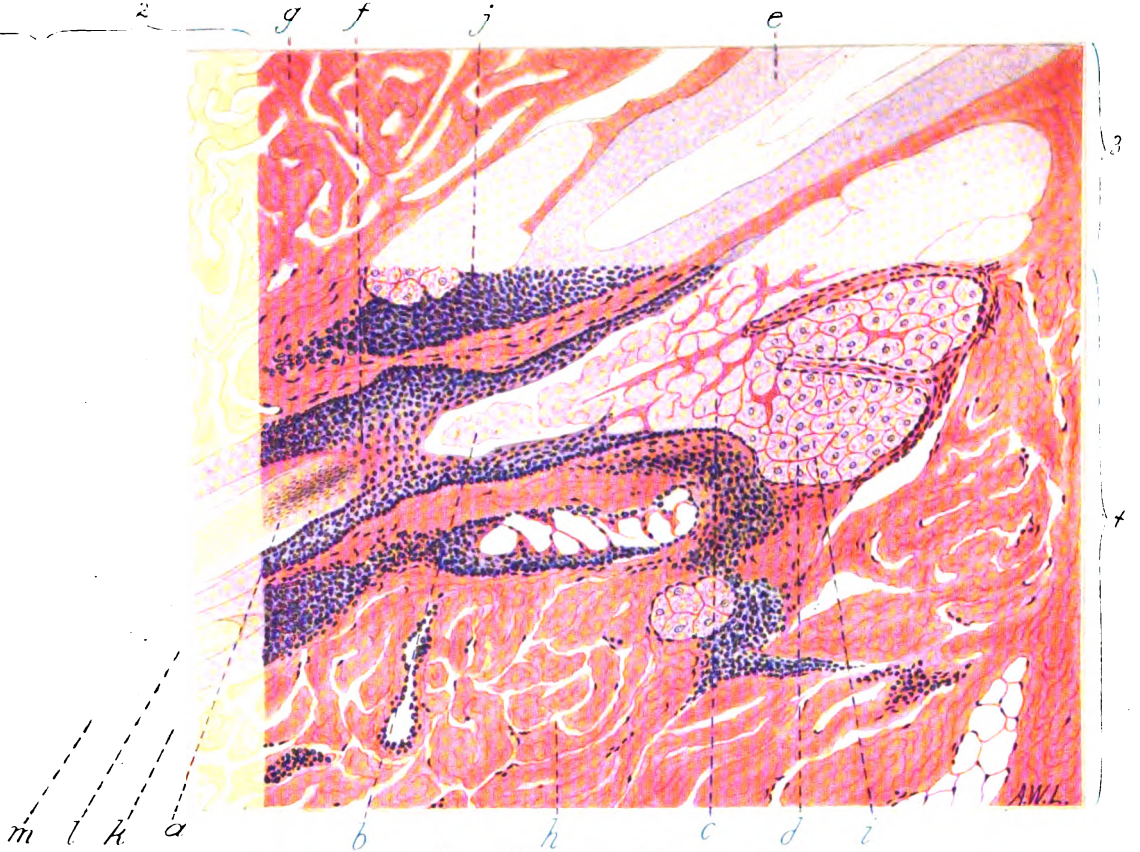

FIG. 17.—Sebaceous Gland from Human Scalp, enlarged about 100 times.
Drawn in steps to illustrate the use of colors.

discharged brush as described in the use of black pigment, Fig. 11. Accordingly, these colors should be prepared somewhat darker than the tints desired, since all of the pigment will not remain upon the drawing when the excess is removed.

The *stage of detail* here is much the same as described for marking drawings in black and white, save that the real colors observed in the tissues are used instead of black or some degree of its intensity.

Occasionally some of the prepared colored drawing inks may be used, and, where this is possible, Higgin's will be found the best; but, the fact that most of these inks are of intense brilliance precludes their common employment, for results thus gained often resemble some style of Chinese art much more than examples of properly stained tissues. Furthermore, dilution of these inks does not satisfactorily soften their color tones. In preparing water-color solutions for the placing of detail, glue or gum-arabic, should be incorporated with them by using the method given under dilution of black pigment and for the same reason.

Since water colors do not rub, there is no need of fixing them.

It should be mentioned, in closing this chapter, that any conceivable combination of graphite, ink, crayon and water color may be utilized to illustrate a microscopical subject; but, since only individuals of skill and training can get anything but erroneous and unsightly pictures with this hybrid method of drawing, the beginner is strongly advised to begin and complete his work in one style.

SECTION II.

OUTLINES FOR LABORATORY WORK.

GENERAL INSTRUCTIONS.

Time Required.—The following outlines and directions for laboratory use in histology and microscopic organology cover an amount of work which, in the experience of the author, may be accomplished by the average student, exercising an intelligent economy of his time, in a course allowing three laboratory periods of three hours each per week throughout one school year. The work falls under three general heads, each requiring approximately the same amount of time: (1) The Histology; (2) Microscopic Anatomy, involving the organs comprising all the functional apparatuses of the body except the nervous, and (3) The Central Nervous Apparatus, or system, and the organs of special sense. In some institutions of high rank it is not thought advisable to attempt to cover all the subjects outlined here in the first year, the time being so allotted that the latter part of the work, the gross and microscopic anatomy of the central nervous system and organs of special sense, is offered as a separate course in the second year. There are good reasons for this arrangement, based upon both curriculum and especially upon the development of the student's information and, in such cases, that part of these outlines dealing with the nervous organs may be used as a guide in the work of this second course. In institutions not allowing the full time here required to be devoted to the work, portions of the outline will have to be omitted at the discretion of the instructor.

Nature of the Work.—The work outlined deals wholly with normal tissues and organs. In certain rare cases reference is made to abnormal structures, but this is done solely as a means of aiding the student to get a more concise and permanent grasp of the normal appearances and normal microscopic structure and relationships. A thorough familiarity with the tissues and architecture of the organs of the normal body must, of necessity, precede an ability to study intelligently and distinguish pathological modifications and a comprehension of the functional and structural changes produced by disease.

Preparations of human tissues are frequently called for in the directions for the study of the different subjects. The laboratory in which these outlines have been used, in mimeographed form, during the past years, has been kindly given access to autopsies of executed criminals from time to time, and thus, fortunately, has been able to supply its students with an unusual amount of freshly obtained and properly fixed human material. In laboratories in which normal human material is not available, tissues taken from the monkey and

dog, and, at times, from certain others of the domestic animals, will serve the purpose more or less satisfactorily, for, if properly chosen, such tissues resemble the human quite closely. In addition, they have an advantage from the viewpoint of comparative anatomy. Of the more easily available domestic animals, the author has found that the organs of the dog and hog are more generally identical in structure with the human. For the work involving the histology proper, the tissues of any of the higher mamals will serve in most all cases. Human blood, for example, has distinctive features, but it can always be obtained. In any course, the cat, rabbit, rat, and at times the frog, are called upon as a matter of ease and convenience. For instance, the Pacinian, or lamellated, nerve-corpuscle of the cat is practically identical in structure with that of man, and it is far more easily obtained and better preparations can be made of it if taken from the mesentery of the cat. Furthermore, in the organology, while the chief object of the course outlined here is to familiarize the student with the detailed structure of the human body, the value of comparing the organs of different animals with those of man is recognized as exceedingly helpful toward that object, and it is assumed, even when human material is furnished, that the student will frequently be given preparations from similar organs of other animals. The study must be based, of course, especially by students preparing for the practice of medicine, upon the structure of the adult human body, whatever the variety of the preparations used.

As considered advisable in a course of this kind, the classification of structures and the arrangement of topics is made upon an almost purely anatomical and functional basis, but, every care should be taken to avoid losing sight of the embryological principles and processes giving rise to the structures. To attain an intelligent and useful familiarity with the adult structures, a knowledge of the origins and processes by which the various tissues and organs are developed is very essential, and the student is urged to keep in mind, in every case, the processes by which arise the structures embraced under each of the different divisions of the work. At times, when deemed especially essential, the student is supplied with sections of embryos and preparations of developing tissues and organs in order that the processes of their origin or elaboration may be reviewed or studied in detail.

One of the most common defects in the study of the microscopic structure of the tissues and organs is that the student does not keep in mind their natural color, physical characters, gross appearances, and actual relations in the fresh condition. The mental pictures most often acquired consist wholly of the appearances of the specially treated and stained preparations of the structures with no suggestion of their actual appearance in the body. Furthermore, a section of an organ, of the spinal cord, for example, may be studied and mastered as to its detailed structure without considering its position, plane, or its relations to other parts of the organ, that is, without keeping in mind the shape, extent and relations as a whole of the organ to which the section belongs. For purposes of orientation, and in order that this very usual gap between gross

and microscopic anatomy may be bridged, the directions here given begin with, or at some stage involve, the consideration of the macroscopic appearances of each group of tissues or system of organs. Somewhere near the beginning of the study of each subject, or group of allied structures, a freshly killed dog, cat or rabbit, or material from the butcher, at times is called for for this purpose. To make the most economical use of this material when provided, the student should read over the division of the outlines dealing with the given group of structures, note the parargaphs in which the study of appearances in the fresh are suggested and, in their sequence, should make all such studies called for during the laboratory period in which the fresh material is available. The transition from the macroscopic conditions to the microscopic detail is made with the hand lens, dissecting microscope, teasing methods, free-hand sections, etc. The value of making these preliminary examinations, whenever possible, can hardly be too strongly emphasized.

Again, in the microscopical study of a preparation of a tissue or organ, one is prone to omit the consideration of its structural and architectural resemblances and its relations to other structures. It should be realized that there are no chasms of demarcation between structures, no abrupt changes, in the body. The more familiar one becomes with the various structures, the more one realizes that there may be found gradual transitions between the varieties of tissues and between the structures of organs. The sequence in which the topics of the different subjects are arranged is usually such as to aid in noting resemblances and similarities of structure. The student should strive to become, finally, so familiar with the organization of the body that the structure of one part, called to mind, will suggest that of another and so on, till the entire body may be unwound, as it were, into an unbroken chain.

Let all studies be centered upon the actual tissues themselves. Use the text-books and other reading matter collaterally as a means of becoming able to intelligently observe the preparations. Decide upon the correct answers to the questions asked throughout the outlines. To most of these questions, direct answers are expected; some are asked for the purpose of suggesting observations and lines of inquiry leading to further conclusions.

Preparation of the Material.—The more detailed study is made from specimens prepared by methods of technic designed to emphasize their principal microscopic features. It is intended that the routine of the preparations be prepared by the technical assistant of the department, or by members of the teaching staff, and issued to the student who only mounts them and labels the slides. If the work outlined is accomplished in the time specified, the student will have no time to give to histological technic other than the amount called for in the outlines. Technic, however, is a very necessary part of scientific training and the student is expected to be more or less familiar with its general details from courses taken prerequisite to this course. If not, he should take a special course in histological technic rather than frequently interrupt the necessary consecutiveness of the study of the subjects of this.

another course. In addition, it is not considered advisable for the students to make the routine preparations, for such are never of uniform quality, throughout the class, and most of them are so poor and unsuited that their study largely involves an unwarranted waste of time. However, it is required that each member of the class be familiar with the general methods of histological technic. If he is unable to present proof of this, he is required to present a number of acceptable preparations made in his extra time during the year. Their preparation must include the process of removal, fixation, hardening, embedding in both paraffin and celloidin, sectioning, and staining by two or three of the common methods.

Except where especially thin sections are needed, the routine preparations are issued to the class in the form of celloidin sections. For the reception of these, each student is supplied with a stender-dish with ground-glass cover. Upon both the dish and the cover he must put a label bearing his name. An amount of "clearing-oil" is kept in the dish, so that, in issuing the sections, the assistant at the same time transfers them to this oil where they are cleared ready for mounting by the student with the least possible loss of time. When the sections are all mounted, the dish must be returned to the assistant to receive the succeeding batch of sections. Paraffin sections are usually mounted on cover-glasses and stained and cleared by the assistant, the student, in these cases, bringing a slide with a drop of balsam on it to the assistant who then inverts the cover-glass upon the balsam. The assistant will strive, in supplying the sections, to keep well ahead of the class-work so that the balsam of a batch issued will have time to harden before the preparations have to be studied.

The different methods employed in making the preparations called for in the outlines, are summarized in Section III. For the detail of the technic, the student is referred to the standard text-books of histology, special treatises on technic, or to the original papers describing the different methods in detail.

Labelling and Storing Slides.—Slides of perfect white glass and of medium thickness should be obtained. The thinnest form put on the market is too apt to be broken in routine class-work, and the thickest involves an amount of glass unnecessarily obstructing the light, especially should it contain flaws.

The standard size of slide, and that which is used for all ordinary preparations, has a measurement of 75×25 millimeters (3×1 inches).

Choose a neat square label of pure white paper, and the full width of the slide. There are many slide labels on the market which do not possess these qualities. The left hand end of the slide is preferable for the label from the fact that the right hand is usually employed with the focussing of the microscope while the left hand is used in moving the slide on the stage, and the eye falls more naturally to the hand holding the slide when it is necessary to read the label. In writing the labels, exercise uniformity. The following formula will suffice for most specimens:

(1) The subject (name of tissue or organ).
(2) Source (name of animal).
(3) Locality (region of body or organ from which preparation is taken).
(4) Plane of section, if important. (If not a section, substitute the appropriate word, such as teased, whole mount, smear, etc.)
(5) The method of preparation. (If a special method, known by the name of its deviser, was employed, write merely the proper name, such as "Mallory," "Weigert," "Golgi." If a common method, such as celloidin section, stained with hematoxylin and eosin, abbreviate, as "Cel. H & E.")

Sometimes, in case of the special differential methods, it is well to write on the label a word indicating the special feature shown by the preparation. And it often will avoid considerable trouble to draw a ring or square on the cover enclosing the area of the preparation showing the special feature. This ring or square can be made with drawing pen and water-proof ink.

For storing slides for use, large slide boxes are always preferable. Of these, the cloth-covered box of two parts. with hinged cover, printed index, metal catch, and with 100 grooves for slides, is recommended. With medium thin slides, each groove of this box is capable of holding two slides placed back to back. The cloth-covered box does not split or warp as does the wooden one. The small wooden boxes occasion more delays than large boxes from the fact that one cannot assemble his material so well and, when it is necessary to refer to a certain previously used preparation during the study of another subject, one seldom knows in just what box it is stored. As to the system to be used in storing the slides, it is suggested that they be grouped and arranged in the boxes in the order of the arrangement of the subjects and topics followed in the outlines. Whatever the arrangement, let it be readily usable so that a given preparation may be found with the least possible delay.

Equipment for the Work.—The laboratory is supposed to furnish each student with a locker and key and a set of apparatus which should include the following:

1 Compound microscope and case with several oculars and objectives, including $\frac{1}{12}$ oil-immersion.
1 Dissecting microscope.
1 Balsam bottle containing balsam.
1 Wash-bottle for distilled water.
1 Tumbler.
1 Bunsen burner with rubber gas-tube.
1 Dissecting pan (broad shallow cake-pan).
1 Petri dish.
1 Hand towel (exchanged for clean one when soiled).
1 Piece of small glass rod.
2 Stender-dishes (55 x 25 mm.), with ground covers to match, one to be used as "section dish."
3 Small dropping bottles.
3 Test-tubes.
6 Jars for staining on slide, or 6 100 c.c., salt-mouthed pomade or quinine bottles with corks.

2 100 c.c. Reagent bottles.
6 50 c.c. Reagent bottles.
2 8 c.c. salt-mouthed bottles, with corks, one to be used for glycerin.
2 Sheets of filter-paper.
3 Syracuse watch-glasses.

This apparatus is to be returned at the completion of the course, clean and in as good condition as when received. Reagents may be obtained at need upon requisition from the store-room.

In addition to the above list, the student, to begin with, must provide for himself the following equipment. Other articles will be needed during the course, but these may be obtained as occasion arises.

2 Sheets of Strathmore bristol board.
3 Sheets of "Cap, 2-ply" Reynolds bristol board.
3 Drawing pencils, HB, 4H and 5B.
1 Large soft eraser.
2 Drawing pen-points, Gillott's Lithographic, No. 290, and Lithographic Crow Quill, No. 659.
1 Pen holder to suit.
1 Ruler, graded in millimeters.
1 Bottle waterproof drawing ink (India ink).
6 Paper "blenders."
6 Thumb-tacks.
8 Assorted colored crayons, Faber's preferred.
1 Small drawing-board, 12 x 8 inches.
½ Gross slides (3 x 1 in.), medium thin.
½ Oz. Cover-glasses, 7–8 in. square.
1 Large slide-box.
1 package of slide labels.
1 Package of lens paper.
1 Small camel's-hair brush for manipulating celloidin sections.
2 Medicine droppers
1 Section lifter, medium size, see Fig. 18.
1 Pair cover-glass forceps, *a* or *b*, Fig. 19.
1 Pair small dissecting forceps.
1 Pair small dissecting scissors, Fig. 20.
1 Scalpel, medium size, for dissecting and "scratch erasures."
2 Teasing needles, fine, slender points, *a* or *b*, Fig. 21.
1 Section razor.
The text-book or books recommended and an abundance of clean soft linen or cotton cloth for cleaning cover-glasses, slides, etc.

To procure other than sufficient material and instruments, and those of the best quality, *is a most unprofitable economy.*

It is advisable to cut the sheets of drawing paper into four equal parts. They can be more conveniently stored and easily protected from soiling in smaller pieces, than in the original boards. At need, pieces of sizes required may be taken from these with as much economy as from the larger boards.

The teasing needles usually on sale are too course and blunt for fine teasing work. The student can make a better article, and at less expense, by procuring a paper of ordinary sewing needles, No. 7, Sharp's, and then whittling out handles to suit of soft wood into which the needles may be forced, eye first, with a pair of wire forceps. (See Fig. 20, *a*.)

Use of Reagents.—Always use sufficient of a reagent but not a wasteful amount, and use a given reagent only for the purpose for which it is intended.

This is especially applicable to the staining of smear preparations, obtaining hematin crystals, etc. When discarding them, throw all solid materials, acids, stains, etc., into the waste-jars provided, not into the sinks. Beware of fluids containing acids or acid-salts coming in contact with metal pieces of apparatus. Not only will the apparatus be injured but the composition of

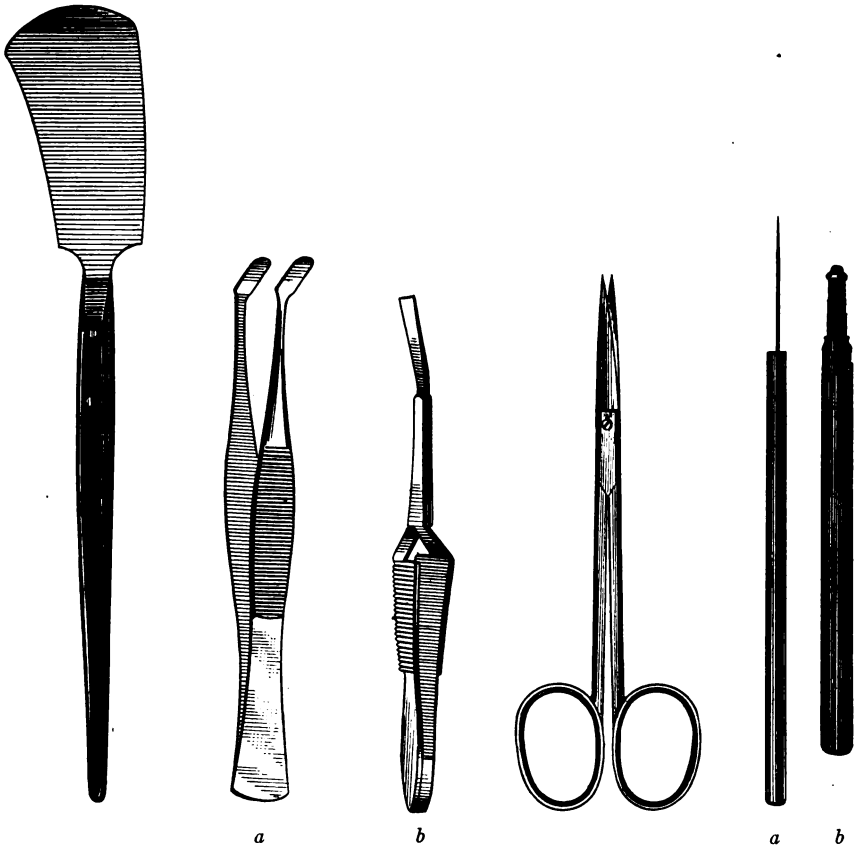

FIG. 18. FIG. 19. FIG. 20. FIG. 21.

FIGS. 18 to 21.—Showing styles of certain of the instruments referred to in accompanying list.

the fluid will be changed, probably causing failure of the expected results. Never place a slide giving off fumes of an acid on the stage of the microscope.

Care and Use of the Microscope.—The student is supposed to be familiar with the general construction and purpose of parts of the microscope from having used it in courses previous to this. Most of the text-books of histology and some of the treatises on histological technic give detailed descriptions of the construction of the compound microscope and the physics of its use, and the Spencer Lens Co., of Buffalo, N. Y., publishes a pamphlet giving its

construction and precautions for its use which is supplied for the asking. It is intended here only to call attention to some of the habits formed and errors quite commonly committed even by those more or less accustomed to the use of the instrument.

During use, it is quite often necessary to clean the lenses. For this purpose, the student is urgently advised to keep on hand a supply of the paper made for this purpose and known as "lens-paper." Use a small piece at a time, and never use the same piece on two occasions for, in the meantime, it may have collected dust or grit. If cloth is used, let it be of soft, perfectly clean, old linen, cotton, or silk; let it be reserved for use on the lenses only; let it be kept in a place protected from grit, and let it be discarded as soon as it is soiled. It is a common habit to draw the handkerchief from the pocket and apply it to a lens. Handkerchiefs are not always clean nor do the contents of the pocket of work day clothes render it a safe place for a lens cleaner. A slight scratch across the surface of a lens is an expensive mishap, and continual, though very slight, abrasions by dusty cloths will eventually render it necessary to send the lenses to the maker for repair. The ordinary worker frequently gets balsam on the objective. This may be removed by dipping the lens-paper or a corner of the cloth in chloroform, absolute alcohol, xylol, or any ready solvent of balsam. Chloroform or strong alcohol are safer from the fact that they evaporate more rapidly and consequently less time is allowed for them to attack the substance with which the lens is cemented in its position. In some cases, balsam itself is used as the cementing substance. After having used the oil-immersion lens during a period of work, always clean off the oil before putting the microscope away, for it will toughen by drying before the next period and occasion more trouble and danger in its removal. Use strong alcohol with the lens-paper for this, followed by dry paper. The process should be repeated with clean paper to remove the cloudy film of water-vapor and oil usually left after the first treatment. Do not allow the alcohol or chloroform to come in contact with other parts of the instrument. The lacquer finish may be dissolved and the metal exposed to corrosion.

Form the habit of being careful in the focussing of the high power objectives lest they come in contact with the slide. Not only may the slide be broken, but the grinding of the surface of the lens upon it is far more to be deplored. A good plan is to remove the eye from the ocular, and, closely observing the objective, run it down till it almost touches the slide, then return the eye to the ocular and focus upward till the field comes into view.

In a cool room especially, the close proximity of the nose and mouth to the barrel of the microscope results in a condensation of the vapor of the breath upon the instrument, often so great that it runs in drops upon the stage. Not only is this an annoyance to the worker, but, if the instrument is not well lacquered, it is conducive to corrosion of the metal. Its continued occurrence, leads to a disintegration of the lacquer itself followed by the defacement of the metal as before. This condensation of the breath may be largely avoided by

fitting a circle of good quality of writing paper around the ocular. The circle may be about two inches wide. The paper quickly acquires the temperature of the breath, which it also deflects from the microscope, and therefore condensation is obviated.

Right-handed individuals should form the habit of using the left eye at the ocular, so that the right eye may more easily be transfered to the drawing on the drawing-board at the right. Otherwise, if the right eye is used for the microscope, the head must be lifted and the nose carried across the ocular in the process of transferring the eye to the drawing, and, in doing this, the nose or cheek often comes in contact with the ocular, fogging it. Or, if the ocular is not touched, it is frequently fogged by the breath in the passing of the nose over it. Thus the lens-paper or cloth must be frequently produced to clean the ocular before work can proceed. The use of the left eye at the ocular will, therefore, not only save considerable time in the course of the day and considerable annoyance in changing the position of the head, but will greatly decrease the using of the cleaning cloth upon the ocular and thus decrease the possibilities of injuring the lens.

By far, the greater part of the fatigue experienced in laboratory work is induced through the eyes. With some workers the use of the microscope is a continuous strain in the musculature of the entire optic apparatus. They squint. Do not try to work with one eye closed, but form the habit as quickly as possible of looking into the miscroscope squarely with both eyes open. In a very few sittings, by concentrating the attention upon the object in the instrument, one can learn almost unconsciously to neglect the image formed by the other eye.

Economy of Time.—The author knows of no work at which time can be more abundantly, frequently, and, often unconsciously wasted than in the histological laboratory. To cover the work outlined here in the period allotted, the student must consider seriously the distribution and use of his time. He wastes time in the thoughtless application of histological technic, in the arrangement of his material and apparatus, in a lack of consecutive thinking and procedure, but, most commonly and most of all, does he waste time in a lack of conciseness of purpose in going about his work. He may begin work on a preparation without being sufficiently familiar with the subject to distinguish the normal from the abnormal, artifacts or even débris from actual structure; he will begin a drawing without knowing what to draw; he will uselessly spend a lot of time at the microscope and then be forced to consult the text-book before he can proceed.

First, he should always become thoroughly familiar with what is said in his text-books and lecture-notes concerning a subject before taking up, in the laboratory hours, the preparations illustrating that subject. The laboratory is not the place in which to read the text-book, especially for the first time.

Second, he should always study a preparation thoroughly before attempting to draw anything from it, first, under low power for orientation, topography and choice of a suitable field, and then under high power to become familiar

with the more detailed structure. He should decide what a drawing shall be intended to represent before beginning to make it. Let him beware of artifacts. They are frequently to be found in even the best preparations.

Third, he should always read the laboratory outline well ahead, and, with his preparations before him, accordingly plan his work with the greatest economy of time.

Drawings.—The making of drawings is not anatomy. It is merely a means of learning anatomy. Experience has shown that after taking the pains to carefully portray a structure on paper, that structure in its detail is more permanently fixed upon the mind than is frequently possible by any other method. It is not necessary to draw everything studied; only the more important things. One should study far more than he draws.

All drawings must be concise in character and neatly done, and, above all, they must accurately portray the structures under consideration. Drawings of the kind that may be popularly called "Artistic" are often worthless. Cultivate a sense for form and proportions. Never exaggerate a structure, avoid "patching," and beware of the tendency, toward which many workers are prone, to make a structure appear "more natural" than it really is. In drawing, one learns to practice habits of neatness and cleanliness and astuteness of observation so essential for everyone, but especially for the successful student of medicine.

Never make a drawing to include a larger area of the preparation than is necessary to the purpose in mind. Drawings involving more than is necessary, not only involve a waste of time, but, in the rush of laboratory work, are often less effective and generally poor in desired detail.

For line-drawings, a large number of which will suffice in an ordinary course in microscopic anatomy, the use of ink is, in most cases, much preferable to pencil. Lines made with the pen are not only more precise and effective, but are necessarily more thoughtful from the fact that one naturally ponders longer before putting the pen to paper. First outline in light pencil, correcting as to scale, curvatures, number of lines and details, and then complete the drawing in ink or in process black in whatever shades desired. Pure line-drawings are usually made in undiluted black.

Always choose carefully the field from which a drawing is to be made. Foreign bodies, grotesque features or artifacts produced by the treatment of the specimen, may be overlooked till after the drawing is well underway and then a new field may have to be chosen or, at least, the drawing may have to be discarded. Make it a rule to study a preparation thoroughly before attempting to draw anything. Study it first under low magnification for purposes of orientation, and for areas promising a field suitable for illustration, and second, under high magnification for closer examination and for the more detailed structure. Decide what is normal and what is abnormal in the preparation and what the drawing shall be intended to illustrate, and then decide upon the most propitious magnification under which the drawing may be made. Then the colors

represented in the field may be studied and analyzed, or their color values decided upon.

Finally, let it be noted, that with most student workers it will be found wiser to confine the use of colors to those drawings only in which it is necessary to illustrate color-differences; to the portraying of those structures only whose distinctive characteristics are either their intrinsic color or their color reactions; that is, their special differentiations by the stains employed in the making of the preparations. The majority of preparations are stained merely to facilitate the study of them by intensifying structures otherwise seen with difficulty or not at all. Therefore, in the majority of cases, the color imparted by the stains need not necessarily be reproduced.

For aid in the technic of drawing, read carefully what is given in Section I. While the treatment there is by no means exhaustive, it is hoped that it may be of use in the saving of time and improvement of results.

Collateral Reading.—At the end of the outline for each general subject there is given a list of the original papers dealing with the respective structures considered. These are merely papers chosen from among the far greater number. For many of the subjects, the literature is so voluminous that in the selection of the advisably few for these lists, numerous papers of importance were of necessity omitted. Some of the papers cited are of recent date; some are old but all are the better for it in that they have stood the test of time. Monographs and dissertations published separately, and papers occurring in other than the more usually available journals are as a rule avoided in these lists as probably less easily obtainable by the student, and papers dealing with mammalian, and especially human tissues were given preference. Students wishing to read further are referred to the literature given by the authors of these papers and to the current numbers of the journals for the more recent literature. Many of the publications chosen here are in other languages than English. The best advice the author can give, is read; read all you can whenever you can. It is the only way to become familiar with your subject.

The Papers.—On the completion of the study of each of the general subjects and the drawings necessary for it, the student is required to incorporate his results in a paper which is to be handed in to the instructor for examination and correction. The subjects are so arranged in the outlines that there will be twelve of these papers in all. Each must contain an epitome of the student's laboratory notes, collateral reading, and final observations, illustrated with his laboratory drawings, and each must fully cover the ground. They are not only required for the information of the instructor as to the progress of the student, but chiefly on account of their pedagogical value to the student himself.

As is well known, the writing down of one's impressions is the most excellent means of fixing them in the mind, but, in addition, in papers, the student has that opportunity to assemble his information, to systematize and to summarize it, so necessary for the acquiring of a well-rounded, definite grasp of a subject.

In the arrangement of the material and the writing up of a subject, one will often be impressed with resemblances and relationships of structures not seen before, but the realization of which aids greatly in making his knowledge of the subject consecutive.

In the construction of the papers, it is suggested that the topics involved be arranged in the order followed in the outline for the given subject and that the laboratory drawings be arranged accordingly in their natural sequence. Make abundant use of the drawings to illustrate the text descriptions, referring to special features by means of the letters or the names indicating them in the drawings. Let the text be as brief as possible, making each paper little more

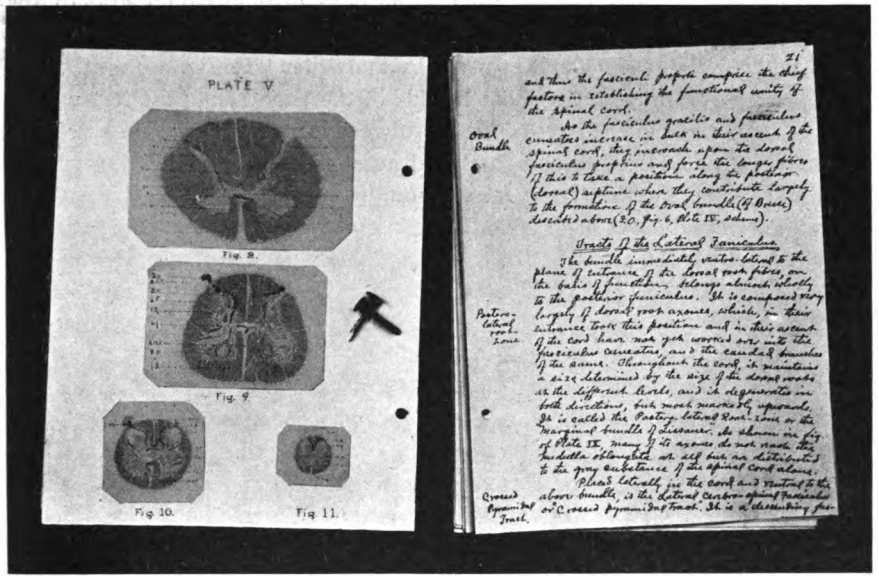

FIG. 22.—Showing the form of paper and paper-fasteners, and suggesting a method of arranging the drawings and text for laboratory papers.

than a summary of a systematized presentation of the detailed characters, relationships, and functional significance of the structures, and the general conclusions of the worker reached from his laboratory observations and corrected from his lecture notes, texts, and collateral reading.

While the work of constructing these papers must be done outside of laboratory hours and is tedious and time-consuming, at the end of the course the student will have his year's work so collected and arranged that in other courses he will refer to it at need in preference to his text-book and with better results, for the pages will bring back to him the actual appearances and studies of the preparations themselves. In actual experiences of a number of years, so universal has been the satisfaction of having done the work, that the

author remembers no case in which a student, at the end, has regretted the time and labor spent.

The writing-paper used for the laboratory papers should be of uniform size and quality throughout the course. Ordinary letter-size (11 × 8½ inches), in separate sheets, unruled, is recommended. It should be obtained with two perforations at the side, as shown in Fig. 22, in order that the pages of each paper may be tied or clasped together. No. 24, "paper fasteners," such as shown in Fig. 22, will be found very satisfactory.

It is more satisfactory, both in construction and to the reader, that one side of the page only be written upon. Always leave a left-hand margin of fair size for convenience of the reader in indicating corrections and for the occasional necessity on the part of the writer of inserting notes of points omitted. In this margin also may be written the topic headings so that they may be readily found both by the reader, and, at need, by the writer (see Fig. 22).

Always mount the drawings on the side of the page facing the text and as near as convenient to the part of the text describing them (see Fig. 22). Let the drawings be referred to as figures and numbered consecutively throughout a paper. When several drawings are mounted on the same page, that page should be referred to as a plate and the plates should be numbered consecutively throughout the course. Number the plates in Roman characters and the figures in Arabics. Then, having the drawings lettered (or the names of the structures attached) and the figures and plates numbered, at any place in a paper, one may refer to a certain detail shown in any drawing by citing the plate, the figure and the reference letter as, for example, "Plate IV, Fig. 2, *a*."

The use of diagrams, schematic drawings and reconstructions are frequently of great convenience and time saving value in describing structures and the interrelationship of the parts of an organ or apparatus, and is strongly advised. If the diagrams required are small, they may be drawn in the text-page as needed. If large and more formal diagrams are required, drawing paper should be used and they should be labeled and mounted and numbered as ordinary figures.

When the paper is assembled, put on a blank page at either side for protection, and write across the front one of these pages the number of the paper and its general title in large characters, thus:

"PAPER VI.

THE RESPIRATORY APPARATUS."

Then put the name of the writer and the date, in ordinary script, at the lower right hand corner and the paper is ready to be handed in.

THE OUTLINES.

(FIRST PAPER.)

I. INTRODUCTORY EXERCISES.

Fabric Fibers. -Cotton fibers, strands of wool from the clothing, shreds of linen, and hair sometimes get caught upon the laboratory preparations. That the student may be able to recognize these when they are found, also as a preliminary test of his powers of observation and ability to use the microscope the following exercises are given:

1. Mount in water on the same slide, a human hair, a few white rabbit hairs and a strand of wool. Examine first under low power and then under high. In what features do they differ?

2. On separate slides mount in the same way a few cotton fibers, some finely separated threads of linen and some strands of silk. Examine them under high power. In what features does the cotton differ from the wool and silk? What is the origin, nature and structure of each?

3. On the same piece of drawing paper, make drawings of short segments of all six specimens. Compare them carefully and enumerate the distinctive features of each.

II. THE CELL.

1. Vegetable.

(a) Clip out a small square from one of the thin membranes between the leaves of an onion, and also, with razor, make a thin longitudinal section of a bit of the thicker onion leaf. Mount both specimens in water and examine. What is the shape of the cell in both preparations, and where are the nuclei situated? Crystals in cytoplasm? Draw one cell from each showing contents of cells and their position with reference to other cells. Functions of the cells as suggested by their contents?

(b) On the same slide, mount in water a small piece of the skin of a potato and also a thin section of the potato. Examine under both low and high power. In what do the cells from the two regions differ as to cell contents? Sketch a cell from each region, showing shape, relative size, and cell contents. Difference between cell-membrane and cell-wall? Under high power sketch a large starch-grain. Explain its appearance and formation.

2. Animal.

(a) Epidermal scales, etc. Mount a drop of saliva. Also on another slide mount in 70 per cent. alcohol, some scrapings from the cheek.

What varieties of cells are to be observed? Are any of them living? Other structures than cells? What are the "salivary corpuscles?"

(b) The living cell. Mount a small drop of hay infusion (furnished). Watch an ameba and sketch it at three or four different intervals. How do cytoplasm and nucleus behave? How does the cell (animal) move? Exoplasm and endoplasm? Explain the method of movements of the free-swimming organisms in the mount. Note particles (organic and inorganic) exhibiting "Brownian movement" and explain it.

(c) Examine under high power stained preparations of amebæ. How do the nucleus and cell contents differ from those of the living cell?

(d) Animal ova—stained.

(1) Sketch a few echinoderm or fish ova in the early stages of division, the 2–cell, 4–cell, and 8–cell stages.

(2) From sections, sketch an ovum in the blastula stage. Do the cells in (1) and (2) differ in size? What is the gastrula and how is it derived? Are there karyokinetic figures observable?

3. Cell Division.—The process.

(a) From stained sections (furnished) of animal ova or of other suitable tissue, draw cells showing the following phases of karyokinesis:

(1) Resting stage of nucleus.

(2) Chromatin filaments in "close skein."

(3) Loose skein.

(4) "Mother aster" and centrosome with polar radiation.

(5) "Daughter asters" and spindle. Explain the appearance of the equatorial zone.

(6) Sketch a cell showing the division of the cytoplasm.

After the division is completed, what is the condition of the nucleus? What changes constitute the prophase, metaphase anaphase, and telophase respectively? Name the six drawings according to the phases represented.

(b) Draw three or four stages of dividing nuclei ("germinal cells") from sections of the neural tube of a mamalian embryo. Is there a cell membrane? Evidences of a syncytium?

(c) Make one or two drawings of the nuclear structures alone from stained sections of the growing root-tips of tradescantia or of the onion, showing in greater detail the behavior of the chromosomes (larger here than in the animal cell).

Discuss heterotypic mitosis as differing from homotypic mitosis. Give reasons for the assumption that mitosis and amitosis are but the two extremes in form of a process, between which extreme all gradation forms exist.

Give the two most probable theories of the origin of the Centrosome. What is indicated by the presence of more than two centrosomes (pluripolar metosis)?

III. EMBRYOLOGICAL DEVELOPMENT

1. Sketch a transverse section of a vertebrate embryo, showing an early stage in the formation of the three germ layers. Designate the chorda, neural tube, anlagen of spinal ganglia, etc. What is the origin of the mesoderm? Carefully fix in mind which tissues of the body are derived from each of the germ layers.

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THE TISSUES.

I. EPITHELIUM.

(SECOND PAPER.)

A. **The simple Epithelia** (with or without cilia).

1. Simple squamous.

(a) Stretch a small area of fresh mesentery with interfitting hard rubber rings or over a piece of cork or glass, and treat with 1 per cent. silver nitrate 1 to 12 hours, keeping in the dark. Replace silver nitrate with glycerin, mount a small piece, spread flat in glycerin on a slide, and expose to diffuse sunlight till silver is sufficiently reduced to show the cell boundaries. Draw a small area of the epithelium (mesothelium) showing the shape and arrangement of the cells. How would they appear in vertical section? Explain the appearance of the cell boundaries. Look for stygmata. How different from stomata? Likewise draw an area of the inner lining (intima) of a capillary contained in the preparation. In what does this "endothelium" differ from the "mesothelium"? From what germ layer are both derived?

(b) Pigmented simple squamous. Remove a bit of the pigmented stratum of the retina from an eye (white rabbit preferable) that has been preserved in formalin. Place in 95 per cent. alcohol, 10 minutes; transfer to clearing oil, 15 minutes; spread flat on the slide, remove oil with filter paper and mount in balsam. Sketch a few adjacent cells showing their shape and characters.
Position of nuclei? Explanation of minute round areas void of pigment granules? Where in the body may be found other pigment-bearing epithelia?

2. Cubic epithelium.

(a) From a stained section of kidney, liver, or salivary gland, draw a portion of a duct showing the characters of this type.

(b) Ciliated cubic. Make an illustration of this type of epithelium from a stained section of a lung. In what passage is it found?

3. Simple columnar epithelium.

(a) Non-ciliated.

(1) Cut a small piece from the pyloric end of a fresh mammalian stomach and place in a dissociating fluid (Ranvier's one-third alcohol, for example) and let it remain one to four days. Gently

transfer a bit of the epithelial lining to a slide, carefully tease with needles, mount in water and gently tap the cover-glass for further dissociation. Examine for isolated cells. Add at the edge of the cover a few drops of an aqueous solution of alum carmine or very dilute congo red. When the cells and their nuclei are differentiated, replace the stain with glycerin and again tap the cover-glass. (*Note.*—To replace a fluid under a cover-glass, place a drop of the required fluid at one edge of the cover and a bit of filter paper at the opposite edge and so manipulate that the required fluid is gently drawn under the cover.) Sketch a few isolated cells and also a small row still associated. General shape of the cells? Where is the nucleus situated?

(2) From one of the villi in a thin stained section of the small intestine make a drawing illustrating the arrangement, character and structure of its epithelium. How does it differ from that of the stomach? Position, nature, and significance of the striated or cuticular border? Include a "goblet cell." What is its structure, function, and origin? Position, function structure, and origin of the basement membrane?

(b) Ciliated simple columnar epithelium. Sketch showing an example of this type either from a stained section of a Fallopian tube, of lung, or of certain of the seminal ducts. What are cilia? Their relation in the anatomy of the cell? Mention three theories advanced to explain ciliary action.

4. Pseudostratified epithelium (usually ciliated).

This type is a transition form between simple and stratified columnar epithelium and may be found in any system of passages containing these two types. Sketch an example from a stained section of the epididymis or from a section of the lung. What produces the appearance meriting the name?

B. **The Stratified Epithelia** (with or without cilia).

1. Stratified squamous.

(a) Pare off a small plate of the outer layer of the epidermis (stratum tum corneum) from the palm of the hand and, holding it in pith, cut with razor thin vertical sections and mount them in water. How are the scale-like "cells" arranged with reference to each other and with reference to the surface of the body? Their structure? Sketch a small group. Replace water with 15 per cent. potassium hydrate and examine again. What action is produced by the KOH? Nuclei? Sketch a few cells as modified.

(b) Make a careful drawing of a stained vertical section of human skin from the volar surface of the finger showing in detail the different

strata composing the epidermis and the characteristics of the cells forming each. "Prickle cells" and "intercellular bridges"? Enumerate the changes giving rise to the stratum corneum. Include the corium in the drawing. Nature of its two layers? Terminal nerve corpuscles in papillæ corii? Other corpuscles found in the tela subcutanea? What is the form of nerve endings in the epithelium proper? Is aquamous epithelium ever ciliated? Types of glands in the section and their origin?

(c) Compare section (b) with a similar section of skin taken from the general surface of the body, and enumerate the differences between it and the skin of the palmar surfaces. In what does the epithelium of the dorsal surface differ from that of the ventral surface of the body?

2. Transitional epithelium.

From a stained section of distended bladder made vertical to its wall, make a drawing of its epithelial lining. How many cells thick? What types of cells are observable? Why the name "transitional?" Papillæ? Compare this section with one from a contracted bladder of the same species and note peculiarities of this epithelium suggested by the comparison.

3. Stratified columnar epithelium (usually ciliated).

(a) Non-ciliated. An example of this variety may be found in a stained section of the oral end of the large excretory duct of a salivary gland.

(b) Ciliated. Draw a small portion of the epithelial lining in a section of the trachea. How many layers of cells? What is their position with reference to the lumen? How do they differ? Goblet cells? Glands? Compare with type found in the epididymis.

(c) Action of cilia. Pith a frog, with scissors detach the lower jaw and with scalpel scrape the posterior portion of the mouth cavity (pharynx). Mount the scrapings in a drop of physiological salt solution and examine under low and then under high power. Note the ciliary activity in both clumps and isolated cells. How is the swimming motion of the small clumps produced? Replace the salt solution with a small drop of 1 per cent. osmic acid. Result and why? Sketch showing cell-outlines and cilia.

C. **Glandular Epithelium.**

(1) Tease and examine a bit of salivary gland or pancreas in the fresh condition. Color? Shape of the cells in the alveoli? Sketch a section of a serous alveolus from a stained section of the submaxillary gland and likewise an alveolus containing mucous

secreting cells. Differences between the two? Striations in either?

(2) From a stained section of the cortex of a kidney draw carefully a few cells of a proximal convoluted tubule? Shape of distal ends of cells? Striations and granules?

(3) From a section of the scalp draw a small area showing the character and structure of the cells of a sebaceous gland. Do all the cells possess nuclei? Describe the functional processes of these cells.

Upon what basis is glandular epithelium classified apart from the preceding varieties? Enumerate the different structural forms and functional varieties of glands.

D. **Neuro-epithelium.**

Draw a taste-bud (calyculus gustatorius) from a section involving the foliate papillæ of a rabbit's tongue or a circumvallate papilla of the human tongue. How many varieties of cells compose it? Distinguishing character of the neuro-epithelial cells proper?

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II. THE SUPPORTING AND CONNECTIVE TISSUES.

(THIRD PAPER.)

A. Connective Tissue Proper.

1. Embryonic.

From a section of a small mammalian embryo (10 to 15 mm. pig), sketch under high power an area of the subcutaneous tissue. It is composed of distinct cells? Why is it called a syncytium? What occupies the interstices? Compare it with the subcutaneous tissue of the adult (human). Differences?

2. **Mucuous connective tissue** or "Jelly of Wharton." What is the appearance and apparent consistency of the fresh umbilical cord? Under high power draw a small area from a stained transverse section of an umbilical cord (pig or human near term), noting the so-called stellate cells and their branching processes. Do all the processes anastomose? May this tissue also be called a syncytium? Structure and analogy of the processes? Structure of the interstitial substance? Origin of fibers? Found in the adult body?

3. Fibrous connective tissue.

(a) *Reticular.*—Examine under high power a stained section of spleen of lymph gland which has been subjected to digestion or to "shaking." Draw a small area showing the meshwork of fine fibers from which the cells have been removed. Note trabeculæ of coarser arrangement. Size of the individual fibers? What determines size of meshes? Where else is reticular tissue found and how does it differ from this? What are the reasons for classifying this variety of fibrous tissue separately?

(b) *White Fibrous.*

(1) Areolar. Take a small mass of subcutaneous or intermuscular tissue from a freshly killed mammal and spread it out with needles on a dry slide into a thin film. Let the edges of the film dry to the slide but keep the center moist by breathing upon it now and then. Put a drop of distilled water on a coverglass and invert it on the center of the film. Examine under high power, noting the wavy and taut bundles of white fibrous tissue of varying sizes and running in various directions; the

single elastic fibers of definite contour and occasionally branching, and also the various forms of "connective tissue corpuscles" in the interspaces, and occasional migratory cells (granular, "mast" cells and ordinary leukocytes). Then remove the cover and replace the water with 1 per cent. acetic acid. What is the effect of the acid upon the white fibrous tissue? On the elastic fibers and the cells? Explain occasional constrictions of the bundles of white fibrous tissue. Next, from a stained vertical section of the skin (human), draw an area of the subcutaneous tissue showing the loosely arrayed bundles of fine fibrils and the so-called connective tissue corpuscles. Are the latter individual cells? Explain their position and appearance.

(2) Tendon, compact white fibrous. Take a small strand of tendon from the tail of a rat, stretch it lengthwise on the slide, letting the ends dry firmly to the slide, keeping the strand straight, but tease the middle slightly in a drop of salt solution. Put cover-glass over teased portion and carefully study the appearances of the fresh tissue. Next, remove the salt solution and replace it with a drop of 1 per cent. acetic acid, and note and explain the effect of the acid and the coming into view of the columns of tendon cells. Then wash off the acetic acid with distilled water, place a drop of carmalum under the cover-glass and let this act till the tissue is well stained. Finally, wash off the stain, and mount in glycerin. Draw an area under high power, showing the fine, wavy, parallel fibrillation of the tendon bundles and the chains of elongated cells with rod-shaped and oval nuclei.

Also sketch a small area of a stained transverse section of a tendon, noting the areolar sheaths of the tendon fasciculi and the irregularly stellate bodies of the tendon cells in the section. What is the real shape of the "tendon cells?"

(c) *Elastic.*

(1) On a dry slide, spread out with needles into a thin film a piece of the fresh intermuscular connective tissue from the animal used above. Make the film to cover the full width of the slide, and let it dry to the slide throughout. When dry, place on the center of the film a few drops of a 1 per cent. solution of magenta made in 70 per cent. alcohol and add a small drop of a 0.5 per cent. aqueous solution of gentian violet. Let the mixture act about two minutes, then drain it off and put on a drop of glycerin and examine at once; or, after draining, let the preparation dry throughout and mount in balsam. Explain the deep staining of the elastic fibers by the alcoholic magenta. By what other

characters may they be distinguished from the white fibrous tissue? Sketch a field including both varieties of fibrous tissue and whatever cells present. What can be said of the size and relative abundance of the elastic fibers? Nature of their sheaths?

(2) On the slide, in a drop of glycerin slightly colored with alcoholic magenta, tease a small shred of the ligamentum nuchæ of the ox. Cover and note the well defined elastic fibers constantly branching and anastomosing with one another. Look for transverse marking on the fibers. Draw, illustrating arrangement.
Also draw a small area of a stained transverse section of ligamentum nuchæ. What is the shape of the fibers in cross-section? What occupies the spaces between them?

(d) *The Neuroglia.*

(1) From a section of the human cerebral cortex, or spinal cord, stained by the Golgi (silver nitrate) method, draw one or two "neuroglia cells." Note the general spider-shape of the cell and the apparent relation of the processes to the cell-body. Varieties? Are they, in reality, cells?

(2) From a section of the human spinal cord stained by the Benda neuroglia method, draw a "neuroglia cell" using oil immersion lens. What is the difference between the tissue when stained in this way and when stained by the Golgi method? What is the relation of the neuroglia fibers to the cells and neuclei? Apply the idea of the syncytium in the development of the tissue. Do all the "cells" studied in (1) above contain nuclei?

(3) From the section used in (2), draw a few of the epithelial cells (ependyma cells) lining in the central canal. Are they ciliated? Actual cell membranes? Do they send out processes? General arrangements? What and where found is the substantia gelatinosa? If the section of the human cord fails to show the ependyma cells well, on account of occlusion of the central canal the spinal cord of the hog or dog may be used instead.

B. **Cartilage.**

1. **"Pre-cartilage"** (embryonic cartilage). Draw a small area, showing an early stage of cartilage as it appears in a stained section of a vertebrate embryo [section used in A, 1, (a) above]. What is the relation of the cells to each other? Shape? What is the nature and origin of the matrix or intercellular substance? Significance of the capsules?

2. **Hyaline cartilage.**

(a) *Articular cartilage.* Cut a very thin tangential section of the cartilage covering the articular surface of the end of a long bone

freshly obtained (from the butcher), taking a section (1) from near the edge of the joint, and (2) one away from the edge. Treat the sections 2 to 4 minutes with 1 per cent. acetic acid, then wash in water, mount in glyerin and examine. What is the shape and arrangement of the capsules and of the cartilage cells situated in the lacunæ? Do the cells fill the lacunæ? Are there any indications of multiplication of cartilage cells? What is the nature of the matrix in the second section? Note the gradation from fibrous connective tissue into cartilage (transitional cartilage) as shown by the presence of connective tissue fibers from the synovial membrane in the matrix of the first section taken. Compare with stained section issued. Make drawings of small areas from each region.

(b) *Costal cartilage.* Under low power, examine a stained transverse section of rib-cartilage (human or dog). What is the relation of the perichondrium to the cartilage? What is the arrangement of the cartilage cells lying just under the perichondrium? What may this arrangement indicate? Are there blood-vessels in the cartilage? In the perichondrium? Draw, showing relations and arrangements. Under high power, draw a small area from near the center of the section. How are the cells arranged? What is the nature of the matrix? Any indications of a fibrillar structure? Why classified as hyalin? If tendency toward ossification is indicated, where? Difference in this respect between human costal cartilage and that of most animals?

3. Fibro-cartilage.

(a) *White fibro-cartilage.*

(1) Make a thin vertical section from a fresh intervertebral disk (dog or ox) or a transverse section from a semilunar cartilage of the knee-joint. Place on the slide and treat with Lugol's solution, diluted one-half, 5 minutes. The iodine stains the cell because of the glycogen in it, but leaves the matrix unstained. Does the capsule show any concentric markings? What is the nature of the matrix?

(2) From a stained section passing longitudinally through the insertion of the ligamentum teres into the head of the femur, make a narrow drawing showing the transition of hyaline articular cartilage into white fibro-cartilage. Under high power, draw a small area of the white fibro-cartilage. Shape of cells and relation of cells to fibers? Endoplasm? Interfibrillar matrix?

(b) *Elastic (yellow) fibro-cartilage.*

From a specially stained section of the external ear (human, monkey, hog or ox), draw, under high power, a small area show-

ing the size and arrangement of the cells and the nature of the matrix. Are both white and elastic fibers present? Are there cells in the process of division? Interfibrillar matrix? What is the color of this cartilage in the fresh state? Why? The external ear of the ox contains coarser fibers than most animals while that of the rat and mouse shows practically no fibers.

C. Bone.

1. Compact and spongy or cancellous bone, **macroscopic appearance.** With the naked eye or hand-lens examine the structure of a long bone (human femur preferable), which has been sawed longitudinally through epiphysis and diaphysis. Make a sketch showing the gross structural difference between the two localities, giving some attention to the architecture of the cancellous bone of the epiphysis.

2. **Decalcified bone.**

From a thin transverse section through the shaft of a small long bone, stained by the Van Gieson method, make a drawing under low power of a narrow segment passing from marrow cavity to periphery. Note (a), the periosteum and fibers of Sharpey and their relation to the bone; (b), the lamellæ of the bone structure, namely, the perimedullary or inner circumferential lamellæ; periosteal or outer circumferential lamellæ; the Haversian systems, and the interhaversian areas. Is the periosteum arranged in layers? How are the bone cells or corpuscles arranged? What is their shape? What constitutes the lamellæ? By what is an Haversian canal occupied? General structure of osseous matrix? What is the arrangement of the connective tissue fibers of the lamellæ?

3. **Macerated bone.**—Ground section.

(a) With a fine saw, cut as thin a disk as possible from the shaft of a long bone which has been boiled in an alkaline solution till nearly white when dry, or one from an ordinary skeleton. Grind it between two hones until thin enough for fine print to be easily read through it. Wash off the débris with water, soak in two changes of absolute alcohol 20 minutes, then in a bottle containing pure ether 12 hours or longer, to extract the remaining oil. Let dry thoroughly, keeping it clamped between two slides to prevent warping. To mount, first heat a small drop of balsam on a slide until, when cool, it is hard enough to be merely dented with the finger nail. Then place the bone section upon the hardened balsam, lay the cover-glass on it, gently warm till the balsam becomes plastic and then firmly press down the cover. Do not heat

so far that the balsam is thin enough to penetrate the bone structure.

(b) Examine under low power and determine the various systems of lamellæ studied in 2 (b) above. What differences in appearance are to be noted? Sketch a narrow segment. Significance of interhaversian area?

(c) Under high power make a detailed drawing of an Haversian system. What are the lacunæ? Canaliculi? Do the canaliculi anastomose? Do they communicate with those of neighboring systems? Examine a longitudinal section and determine the direction and relationship of the Haversian canals, and the direction of the long axes of the lacunæ. Make a sketch illustrating the shape of the bone corpuscle. Differences of its three axes?

4. Development of bone.

From a stained longitudinal section of a limb of a fetal pig of about four centimeters stained by Van Gieson's method, or of a finger of a human fetus, draw under low power the appearance presented by a median section of one of the long bones (now chiefly cartilage). Some of the stages of both the endochondral and the intramembraneous form of bone development may be observed in the same section. Near the middle of the shaft or diaphysis note the locality of the center of ossification with its primary medullary spaces and different forms of cells. Note the transition of the perichondrium into a periosteum with outer fibrous and inner thick, osteogenetic layers. Look for ingrowths of osteogenetic tissue (periosteal buds). From the epiphysis toward the center, note the characteristic changes in the shape, size and arrangement of the cartilage cells. Identify "calcifying cartilage matrix." Under high power identify "osteoblasts," and "osteoclasts." Draw small areas showing each in their positions. Also draw illustrating the intramembraneous bone formation taking place under the periosteum. By which process is the shaft of the bone formed? The epiphysis? Is cartilage transformed into bone or replaced by bone?

D. Adipose Tissue.

1. From the subcutaneous connective tissue in the stained section of the skin previously used, or from any of the previous preparations containing them, sketch a few fat cells showing the position of nucleus and cytoplasm. In this section and in the section of fetal material used above, look for cells in the process of fat formation. Describe the process from its earliest stage to the fully developed fat cell. Origin of the cells? What changes occur during emaciation?

2. On a dry slide, spread with needles a film of fresh intermuscular tissue containing fat. Upon the center place a drop of 1 per cent.

osmic acid and let it act 10 minutes. Action of the acid? Why? Its use as a test in pathology?

3. To another fresh film, or one taken from a specimen preserved in formalin, add a drop or two of saturated solution of Sudan III in 70 per cent. alcohol (or Scharlack R.). Let this act 10 minutes, wash off with 70 per cent alcohol, stain 10 minutes with dilute hematoxylin, wash again, mount in glycerin, and examine under both low and high power. What is the peculiar action of the Sudan III (or Scharlack R.)? Its use as a test in pathology? What is stained by the hemotoxylin?
4. Study a digested preparation which contained adipose tissue. What is the nature of the capsules or cell membranes? Draw one or two under high power.

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E. BLOOD AND LYMPH.

(FOURTH PAPER.)

1. General composition of blood.

(a) Thoroughly clean a slide and cover-glass. With a sterilized needle or spring-lancet prick the ear or the end of the finger which also has been just previously bathed with ether-alcohol and thoroughly dried. Place a small drop of blood on the slide and quickly put on the cover. Examine at once. How many and what are the general morphological constitutents? How are the red corpuscles (erythrocytes) arranged? Explain the arrangement. Does it occur in the circulation? Tap the cover with needle and observe the colorless corpuscles sticking to the slide. Which variety of corpuscles is the more numerous? Watch an individual white corpuscle. Does it change shape? Ameboid movement? Elementary corpuscles or blood platelets? Shape? Fibrin filaments? Sketch one of the rouleaux of red corpuscles and three or four white corpuscles. Do the corpuscles vary in size?

(b) Make another mount by adding fresh blood to a drop of Toisson's solution on a cover-glass. Sketch a red blood corpuscle lying flat, and also one in profile. Shape? Central pallor? Nucleated? Measure 20 red corpuscles and 20 white, and determine the average diameter of each variety.

Note.—For measuring objects under the microscope, take a "stage micrometer" having on it one millimeter ruled into hundredths, and with it, carefully determine the value of the spaces or divisions of the arbitrary scale ruled on an "ocular micrometer." The dimensions of the spaces of the stage micrometer being known, the size of the object, which, with a given magnification will occupy one or more divisions of the ocular micrometer, is easily calculated. With draw-tube at a given position, determine the value for both a high and a low power objective. The unit of measurement is the micromillimeter, or $\frac{1}{1000}$ of a millimeter.

(c) From the slide used above, also sketch a "crenated" red corpuscle. What is the cause of the crenation? The crenation illustrates a pathological form observed in "Maragliano's degeneration." In a corpuscle which has not undergone crenation, note the enlargement of the central pallor (endo-globular degeneration). Why?

2. **Blood platelets** (elementary corpuscles). Stained preparation. Cleanse the finger, place on a small drop of a freshly made 1 per cent. solution of methyl violet in normal salt, and prick the finger through the drop so that, as the blood exudes and mixes with the stain, the plate lets are stained. Mount and examine under high power. Sketch a few platelets showing shape and relative size. What are they? Tendency to collect in clumps? Note the white blood corpuscles which are also stained, and the shape of their nuclei.

3. **Fibrin.** Stained preparation.

On a carefully cleaned cover-glass, draw a drop of blood, and immediately spread it out thin. Put aside in a moist chamber (invert a wet watch glass over it) 15 minutes to coagulate. Then let dry at the edges and carefully place in a watch glass of distilled water for 5 to 10 minutes. Drain off the water, place in 70 per cent. alcohol 5 minutes or longer, drain, and then put on a drop of 1 per cent. acqueous methyl violet. Let this act 5 minutes, rinse with water, and treat in a similar way with 1 per cent. eosin. Again rinse in water and let the preparation dry thoroughly, and mount in balsam. Sketch a small part of a field showing arrangement of fibrin filaments, etc. Are blood platelets more numerous than in 2, above? Why? Relation of fibrin filaments to clumps of platelets?

4. **Blood pigment.**

(a) To a mount of fresh blood, add, at edge of cover-glass, a drop of distilled water and examine under high power. What is the effect of the water on the shape, size and color of the red corpuscles?

(b) To a mount of fresh blood add a drop of 1 per cent. tannic acid and cover. What action does the acid have upon the coloring matter of the corpuscle? Why? Sketch, showing effect.

(c) *Blood crystals.*

(1) *Hemaglobin crystals.* Put a large drop of mammalian blood, preferably that of the rat, on the slide, and let dry without covering. When dry, add a drop of distilled water and cover. Let dry and examine. When dry, the preparation may be mounted in balsam. What as to the sizes, shapes and color of the crystals? Sketch a few. Why was the water added?

(2) *Hemin crystals.* Put a large drop of human blood on the slide and let dry thoroughly. Then place on it a small crystal of sodium chlorid and a drop of glacial acetic acid. Stir with a clean glass rod for about a minute and then dry by holding the slide high above a small Bunsen flame. Examine, and if unsuccessful,

repeat the application of the acid and the stirring. When successful, dry thoroughly and mount in balsam. Examine under high power. How do the hemin crystals differ in form and size? Color? How do they differ from hemaglobin crystals, and from the crystals of sodium chlorid? Sketch a cluster and one or two lying singly.

5. White blood corpuscles.

Two consecutive hours are necessary to make preparations by the Ehrlich method for the study of the different kinds of white corpuscles. The steps in this method are as follows:

(a) Put a Bunsen flame under the tapered end of the copper bar, furnished, and, when hot, without removing the flame, clean the bar thoroughly.

(b) When the bar has been heating fifteen or twenty minutes, or till equilibrium is established between the radiation and the absorption of heat, run a thin stream of water along it toward the flame, and mark the point at which the water boils.

(c) Thoroughly clean four cover-glasses. Get a film of blood on each by enclosing a drop of blood between two covers and carefully drawing them apart. Place the covers, film side up, on the bar in line about three-fourths of an inch nearer to the flame than the line at which the water boils. This will expose them to a temperature of about 120° C. Protect them from dust and let them remain subjected to the heat for at least one and one-half hours. An oven with thermometer may be used for the fixation.

(d) The "fixation by heat" complete, remove the covers, lay them level, film side up, and cover the films with "Ehrlich's triple stain." When this has acted 10 to 15 minutes, rinse off the surplus stain by holding each cover in forceps and dipping it through distilled water 3 or 4 times. Then dry the preparation thoroughly by holding it, edge downward, high above the flame and, when dry, mount in balsam on the slide. The washing through water should cease when the greater part of the film appears a light reddish orange by transmitted light.

(e) In a similar way prepare two other films and fix them in ether-alcohol ½ to 2 hours. Then rinse consecutively in 95 per cent., 80 per cent. and 50 per cent. alcohol and then, in a closed vessel, stain from 2 to 24 hours in dilute Ehrlich's hematoxylin to which 0.5 per cent. of eosin has been added. Wash with water, dip through 70 per cent. and then 95 per cent. alcohol, dry or clear, and mount in balsam.

From these 6 preparations distinguish and sketch the following five varieties of white corpuscles (Ehrlich's classification):

(1) Small mononuclear (basophil cytoplasm with no granules).
(2) Large mononuclear (basophil).
(3) Transitional form (small neutrophil granules).
(4) Polymorphic nuclei (neutrophil) and
(5) Eosinophil.

What is the color of the red corpuscles? Why? Are there nucleated erythrocytes? What are myelocytes?

(f) Many methods of staining and fixing white blood corpuscles are in use, but descriptions of the results obtained with any are usually based upon Ehrlich's classification and the appearances obtained with the Ehrlich method. A more rapid method and one more readily, and quite generally, applied in practical work is Wright's modification of Leishmann's method. With this method, the films, may be made in the usual way, whenever convenient, and allowed to dry in the air, and then, if kept dry and protected, they remain stainable for days or even weeks. This method is as follows:

(1) Make cover-glass films of blood as in (c) above, and let dry at room temperature.
(2) Using one film at the time, pour 2 or 3 drops of Wright's stain on the film and allow it to act alone, one minute.
(3) Then add, to the stain on the film, distilled water, drop by drop, till the mixture becomes translucent and a delicate, yellowish, metallic scum forms on the surface. Do not dilute until the stain becomes transparent. Let this mixture act two or three minutes.
(4) Decolorize by dipping through distilled water till the film in its thinner portions appears a pinkish orange by transmitted light, with practically no bluish tinge. It is blue at first. The color of the thicker portions is disregarded.
(5) Remove the surplus water by draining and light application of filter paper, dry at room temperature, or by gently warming, and mount in balsam on the slide.

6. Comparative.

Make cover-glass preparations as in 5 (e) of both frog's blood and bird's blood. Study the red and white corpuscles carefully and determine in what features they differ from the mammalin (human) and from each other. Sketch a few red corpuscles from each preparation.

7. Determination of the number of blood corpuscles.

(a) *Of red corpuscles.*

Of the many devices for blood counting, the Thoma-Zeiss hemocytometer is chosen as the most practical and accurate. Ask for

instruction at all doubtful points and exercise especial care in the following:

(1) In cleaning the pipettes.
(2) In cleaning the counting chamber.
(3) In diluting the blood.
(4) In mixing, as the blood is drawn, after it is drawn, and before each count.

Dilute with Toisson's solution. One part of blood to 200 parts of fluid will be found a workable ratio for normal blood.

Formula for the determination of the number of red corpuscles with Thoma-Zeiss apparatus.

Let X = number of red cells per cu. mm., the unit volume usually employed.
Let Y = total number counted in given number of squares (N).
Let N = number of squares counted (usually 200 squares).
Then $\frac{Y}{N}$ = average number of corpuscles per square.

Each square has sides of $\frac{1}{20}$ mm. and, therefore, has an area of $\frac{1}{400}$ sq. mm. Then, number in 1 square multiplied by 400 gives number in 1 sq. mm.

$\frac{1}{10}$ mm. = thickness of film in counting chamber. Therefore, $\frac{Y}{N} \times 400$ (area of film), multiplied by 10 gives the number in one cu. mm. of diluted blood.

$\frac{1}{200}$ = the dilution. Therefore, the last result multiplied by 200 will give the actual value.

Thus, $\frac{Y}{N} \times 400 \times 10 \times 200 = X$, or $4000\ Y = X$.

Make several counts. If results are approximate take the average. Discard all doubtful results.

(b) *Of white corpuscles.*

Use same apparatus as for red cells, but use pipette indicated for white cells. Exercise the same care as for red cells. Use 0.5 per cent. acetic acid for the dilution. Why? Dilute 1 part of blood to 20 parts of the acid. Why? Use same counting chamber as for red cells but use entire field of $\frac{1}{5}$ or $\frac{1}{6}$ objective as area to be counted. Adjust draw tube till the field is a circle with 10 squares of counting chamber, or $\frac{10}{20}$ of a millimeter, for its diameter. Then its radius will be $\frac{5}{20}$ or $\frac{1}{4}$ mm., and the area of the field to be counted is the area of the circle.

Formula for the determination of number of white corpuscles.
Let X = number of cells per cu. mm.
Let Y = total number counted in given number of fields (N).

Let N = number of fields counted (usually 20 fields).

Then $\frac{Y}{N}$ = average number of corpuscles per field.

$\frac{1}{10}$ mm. = thickness of film. Therefore, average number of corpuscles in areas counted, or $\frac{Y}{N}$, multiplied by 10 = average number in a field having a thickness of one millimeter.

$\frac{1}{200}$ = dilution. Therefore, average number counted multiplied by 200 = actual value.

π times square of radius = area of circle. $\pi = 3.1416$.

Then, $\frac{\frac{Y}{N} \times 10 \times 200}{\pi \times R^2} = X$ or $51.02\ Y = X$.

8. Estimation of hemoglobin.

The Gow hemoglobinometer as modified by Sahli will be supplied.

(a) The standard color tube of picrocarmin solution corresponds to the color of a 1 per cent. solution of normal blood in distilled water. Why distilled?

(b) The blood is drawn in the capillary tube graduated for 20 cubic millimeters.

(c) The graduated test tube is for diluting and measuring the diluted blood.

With clean medicine dropper, put a few drops of distilled water in the graduated test tube. Draw the 20 cu. mm. of blood and after wiping off the tip of the pipette, force the blood into the water in the test tube. Wash pipette with distilled water 2 or 3 times, forcing the washings into the test tube. Then, drop by drop, add more distilled water to mixture in test tube till the color against white paper held to the light corresponds to the color of the standard solution held likewise. Then read. If the reading is 100 on the graduated test tube, then the amount of hemoglobin is normal. If above or below, the percentage above or below normal may be read.

9. Blood formation.

(a) In a section of a mammalian embryo, note the form of the red corpuscles in the blood-vessels and in the liver or spleen of the embryo. Sketch a few showing their character and variations.

(b) Red bone marrow. Cut off the sternal end of a rib or the epiphysis of one of the long bones of a freshly killed animal (dog); with strong forceps squeeze out some of the red marrow and make two cover-glass smear preparations as for blood. Fix for ½ to 2 hours in ether-alcohol. Transfer to 95 per cent. alcohol, then to 70 per cent. alcohol, then to water, five minutes in each, and stain in

hematoxylin and eosin for twenty minutes. Wash, dehydrate in alcohol, clear in Xylol and mount as in 5 (e). Look for nucleated erythroblasts, normoblasts and ordinary red corpuscles. Relative abundance of hemaglobin? What is the fate of the nuclei? Eosinophilic leukocytes? Myelocytes and myeloplaxes? Explain the latter. Evidences of cell division? Sketch an area showing a number of the forms of red cells identified.

The spleen and lymph glands may be also considered in connection with blood formation. White blood corpuscles are especially abundant in them and may be observed in the process of division.

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III. THE MUSCULAR TISSUES.

(FIFTH PAPER.)

A. Smooth or Non-striated Muscle.

1. Remove a shred of one of the muscle layers of a bladder which has been fixed in 33 per cent. alcohol. With needles tease it apart in a watch glass of the alcohol, and then replace the alcohol with enough dilute Delafield's hematoxylin to cover the tissue and let the stain act 15 to 20 minutes. Wash out surplus stain with 70 per cent. alcohol, and replace 70 per cent. with a few drops of a 1 per cent. alcoholic solution of eosin for 5 minutes. Wash out eosin with 95 per cent. alcohol and replace 95 per cent. with "clearing oil." After clearing 10 minutes, transfer a small shred of the tissue to the slide and carefully tease the muscle "cells" apart. Drain off surplus clearing oil, mount in balsam and examine under high power. What is the shape of the muscle cell? How many nuclei? Their shape and where situated? What is the structure and general arrangement of the cytoplasm? What is the "anisotropic substance?" Is there any difference in cytoplasmic structure in the immediate vicinity of the nuclei? Is there a cell membrane? Intercellular cement? Draw two or three cells.
2. Study a thin stained section of the tunica muscularis of the stomach or intestine. What differences are to be noted between this smooth muscle cell and that from the bladder? Are there any indications of intercellular bridges? Make a careful drawing of a small group of cells in transverse section, illustrating the intimate structure of the cell and intercellular relations. What is the natural shape of the fiber in transverse section? Also, from the same preparation, make a careful drawing of a cell cut longitudinally showing the nucleus and its immediate vicinity and the muscle fibrillæ. Where in the body are the longest smooth muscle cells?

B. Striated muscle.

1. *Cardiac muscle.*

(a) Carefully tease a small shred of heart muscle which has been subjected to the dissociating action of 0.1 per cent. chromic acid. In a watch glass, stain in picrocarmin 2 to 24 hours, wash in 70 per cent. alcohol, dehydrate, clear, and mount in balsam. How does this "cell" differ from those previously examined? Position and number of nuclei? What is the shape of the cell and its

relation to other cells? Note separated muscle fibrillæ (sarcostyles). Staining character of the intercellated disks or lines of junction between cells?

(b) From a specially stained (Kolossow's method) thin section of heart muscle make a drawing of 1 or 2 cells showing their shapes, structure, and interrelations. Note carefully the arrangement of the anisotropic substance resulting in the two forms of striation. Cement substance between fibers and cell membranes? Does a branch of one fiber always join a branch of another? Structural characters of the intercellated disks or lines of junction of cells? Fibril bundles, their arrangement in cross-section, and the rôle of Krause's membrane in them? Consider the peripheral membranes, connecting Krause's membranes, along the sides of the fibril bundle, as a sarcolemma and note the effect of such a consideration upon the conception of individual cardiac cells. From the studies made of smooth and cardiac muscle, what may be said of evidences that the original syncytium, from which they are derived, is maintained?

2. *Skeletal or "voluntary" muscle.*

(a) Fresh. Remove a small shred of an extended skeletal muscle of a mammal which has been chloroformed several hours. Tease finely on the slide in a drop of normal salt, cover and examine. What is the color of the tissue? What can you observe as to the shape and appearances of a single muscle fiber ("cell")? Now remove the cover, replace the salt solution with a drop of 1 per cent acetic acid, let this act 5 minutes, remove it with filter paper, and mount in glycerin. Under high power distinguish the more evident anisotropic elements (fibrils or sarcostyles) surrounded by the "isotropic" substance (sarcoplasm). Number of nuclei in each fiber? Where are the nuclei situated? Look for demonstrations of the sarcolemma where fibers have been compressed by the needles in teasing, and also for the end of a fiber. Sketch a short segment of a single fiber. What is the sarcolemma? Compare the cell with a syncytium. Measure the diameter of 10 fibers and determine their average thickness in micromillimeters. What length may they attain?

(b) Areas of Cohnheim. For comparison, use stained transverse sections of striated muscle from the human subject, the frog, and an insect (hydrophilus). From each of these (under high power) sketch a transverse section of a single muscle fiber choosing those with the most apparent grouping of the sarcous elements or fibrillæ (muscle columns) in the sarcoplasm. What is the relation of the components of these areas of Cohnheim to the transverse and

longitudinal striation of the fiber? What are the chief differences to be noted in the structure of the muscle from the three specimens? What is the most probable significance of the areas of Cohnheim? Include endomysium and sarcolemma in sketches. Make a low power sketch illustrating the arrangement of all the muscle envelopes from epimysium to sarcolemma.

(c) Muscle fibrillæ and their relations to the striations. From thin longitudinal sections of relaxed (extended) mammalian muscle, sketch a few fibrillæ showing the arrangement of the anisotropic and isotropic substances (fibrillæ and sarcoplasm) into the following four bands (disks of entire fiber) composing a "muscle segment."

(1) The large transverse anisotropic disk, (Brücke's line) divided by the more narrow, more isotropic, Hensen's disk (median membrane of Heidenhein).

(2) The thicker, isotropic disks, one on either side of the transverse anisotropic disks.

(3) The intermediate disk or membrane of Krause, separating the isotropic discs and forming the boundaries between adjoining segments.

(The accessory disk of Englemann and the resulting terminal disk of Merkel are described for insect muscle, in addition to the above four.)

In case of contracted muscles, Hensen's disk does not show. Why?

(d) The blood supply of muscle. Remove a bit of injected muscle which has been preserved in alcohol or formalin, dehydrate in 95 per cent. alcohol 5 minutes; transfer to clearing oil, 5 minutes; tease on the slide (not too finely) and mount in balsam. Under medium magnification sketch a few fibers showing the distribution of the blood capillaries among them. How is the muscle nourished directly, and supplied with oxygen?

(e) The relation of muscle to tendon. From a longitudinal section passing through the line of junction between muscle fibers and tendon fibers, sketch 2 or 3 muscle fibers showing the nature of their insertion and attachment to the tendon. How do the fibers end? Significance of the increased number of nuclei? Intimate character of the attachment?

(f) The nerve supply of the muscle. From a preparation of mammalian muscle stained with gold chlorid or methylen blue, sketch a motor-nerve termination. How is the substance of the muscle cell modified in the "end-plate?" Note branching of the nerve fiber. Look for sensory nerve termination or "neuromuscular spindles." Their difference and the difference of the muscle fiber they involve? The many other forms of nerve terminations will be treated under the "sense-organs."

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IV. THE NERVOUS TISSUES

(SIXTH PAPER.)

A. **The Nerve Cell** (cell-body of the neurone).

1. **External Morphology.**—From sections of silver (Golgi) preparations, make drawings accurately illustrating the differences in shape, size, and detailed contour of the following six types of the neurone:
   (a) "Pyramidal cell" from the motor gyri of the celebral cortex (human).
   (b) Motor cell from the ventral horn of the human spinal cord.
   (c) Typical cell from the spinal ganglion.
   (d) Purkinje's cell from the cerebellar cortex.
   (e) Either a "basket cell" or a "granule cell" from the cerebellum; and,
   (f) A cell-body of a neurone from a sympathetic ganglion.
   (Silver preparations of the adult spinal ganglion and of the sympathetic neurone are, as a rule, more difficult to obtain than the other varieties mentioned. The human or pig fetus, or the rat or rabbit at birth, will prove more fortunate for the spinal ganglion. The more difficult method of the so-called "intravitam" application of methylen blue may have to be resorted to with the sympathetic ganglion, and this method, when successful, is also excellent for demonstrating the external morphology of the spinal ganglion neurone. Cajal's method for neurofibrillæ, often results quite favorably with both.)
   What is the function of each of the above types? How do they differ as to the abundance and arrangement of dendrites? What are the gemmules or "pin-head processes?" In what does the axone differ from the dendrites? Localities, peculiarities, and significance of collaterals?

2. **Internal morphology** of the cell-body of the neurone.
   (a) From a transverse section of the cervical region of the spinal cord and from sections of a spinal ganglion, both of which have been prepared by the "Nissl-Held method," make a careful drawing of one of the larger cells of the ventral horn and of one from the spinal ganglion. Note nucleus and nucleolus, their anatomy, position and size as compared with those of the cells of other tissues. Cell membrane and cell capsules? The occurrence, distribution, and significance of the clumps of *chromatophile*

granules? How may the axone be distinguished from the dendrites? Enumerate the differences between the two types of neurone. Note the general shape of the entire section of the spinal cord and that of the areas of white and gray substance. What is the relation and connection of the spinal ganglion to the spinal cord?

(b) Study the "*neuro-fibrillæ*" in a large ventral horn cell or pyramidal cell stained by the method of Cajal. Their extent, distribution, and significance? Make a careful drawing, under high power, showing them in their relative size and relation to the cell processes and the nucleus.

3. The axone or nerve fiber (neuraxis of the neurone).

(a) The medullated axone.

(1) Remove a piece (5mm. long) of one of the spinal nerves of the frog, or of a mammal, and tease it finely on the slide in a drop of normal salt. (The long nerve roots lying in the lumbo-sacral portion of the vertebral canal are best for teasing in that the fibers composing them are less invested with connective tissue.) What is the appearance of the fresh nerve? Now replace the salt solution with a drop of 1 per cent. osmic acid for 30 minutes. What is the action of the acid? Wash with water, mount in glycerin, and examine. What is the structure blackened by the osmic acid? Neurilemma? Sheath nuclei? Nodes of Ranvier? Draw a segment including a node.

(2) Silvered nerve. While the osmic acid is acting upon preparation (1) above, remove another piece of spinal nerve from the animal, tease it slightly on the slide in a drop of 1 per cent. silver nitrate, then place it in a corked vial containing a small amount of this solution, and put away protected from light for from one to twelve hours. Then tease further in a drop of glycerin on the slide, mount in glycerin and expose to diffuse sunlight 20 to 30 minutes. Under high power draw a segment of a nerve-fiber showing its general appearance and the "cross of Ranvier." What are the components of the cross? To what appearance in (1) does it correspond? What part of the fiber constitutes the longer and heavier limbs of the cross? Fromman's lines? Neurilemma? Nuclei? Appearance of myelin sheaths?

(3) Sections. First, from a paraffin mount containing both transverse and longitudinal sections of a medullated nerve stained with cosmic acid and acid fuchsin (Kupffer's method), make drawings, first of the entire transverse section under low power, showing epineurium, perineurium, endoneurium, sketching in a few nerve-fibers with blackened myelin sheaths; and second, draw one or two fibers under high power showing sheath, neurilemma

and detailed structure of the axone. What is the neuroplasm? What is the relation of the axone to the nerve-cell? What are the segments of Lantermann in the sheath? Situation and significance of sheath nuclei? Are the nerve-fibers of uniform size? Measure ten in transverse section and determine the average diameters of both sheaths and axones.

Next, from sections stained by the Benda neuroglia method, study in detail the framework of the myelin sheaths. Draw a transverse section of a medullated axone and also a small portion of a longitudinal section including the region of a node of Ranvier. Relation of the neurilemma and sheath nuclei to the frame-work? Behavior of framework in the formation of the node? Explanation of Lantermann segments?

(b) The non-modullated axone (Remak's fibers). Tease a piece of sympathetic nerve, or a piece of the vagus, previously treated with cosmic acid. Observe between the medullated and therefore blackened fibers, numerous unblackened fibers. What is the nature of the sheath of the latter? Are there sheath nuclei? Nodes? Are there any indications of partial medullation? Do the fibers ever branch? Draw several sympathetic fibers showing varieties and characteristic differences from the medullated. Non-medullated fibers are the outgrowths of cell-bodies situated where?

4. **Nerve terminations.**—Review nerve terminations upon muscle fibers, and Meissner's (bulboid) corpuscles as found in papillæ corii of the skin. Draw a Pacinian (lamellated) corpuscle from a stained preparation made from the mesentery of the cat, and compare it carefully with a "Gential" corpuscle (demonstration preparation). What is the general difference between motor and sensory nerve terminations? Between the so-called "free terminations" and the encapsulated? Compare a neurotendinous nerve ending (demonstration preparation) with the neuromuscular variety. What is the behavior of the medullary sheath upon the approach of the termination of the axone?

Nerve terminations will be further studied with the organs of "special sense."

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MICROSCOPIC ANATOMY OR ORGANOLOGY.

I. THE CIRCULATORY SYSTEM.

(SEVENTH PAPER.)

A. The Blood Vascular Apparatus.

1. The blood-vessels.

(a) Arteries.

(1) From a stained transverse section of a medium-sized human artery (femoral or carotid), draw in detail a segment showing the various tunics. How many and what fundamental tissues enter into the structure of each? How many cells thick is the endothelial lining of the *intima?* Note carefully the elastic (fenestrated) membranes limiting this tunic. Are they continuous? What can be said as to the extent and variety of muscular tissue in the *media?* Does the adventitia, or *externa*, permit of subdivision? Compare this tunic with the media. Note *vasæ vasorum.*

(2) Draw a small segment from a stained transverse section of the aorta, giving special attention to differences between its structure and that of the medium-sized artery.

(3) From a section of aorta which has been treated with a special elastic-tissue stain (orcein, for example), make a drawing of a small segment showing the relative abundance and arrangement of the elastic tissue in the different tunics.

(4) *Fresh tissue.* From a freshly killed mammal, remove a small piece of a medium-sized artery (femoral or carotid), split it longitudinally, and place one piece in 1 per cent. silver nitrate and the other in 25 per cent. caustic potash.

(i) After the first piece has remained about 25 minutes in the silver nitrate, transfer it to a slide in a drop of glycerin, scrape off some of the intima, then cover the whole and expose to diffuse sunlight. When the silver is sufficiently reduced, draw a small area under high power, showing the relative size, shape, and arrangement of the endothelial cells.

(ii) After the second piece of artery has remained in the caustic potash for one to two hours, lay it flat on the slide, and, holding it with a needle, gently scrape both sides sufficiently to lay bare from both sides portions of a "fenestrated membrane."

Cover and examine. What is the nature of the membrane when seen on the flat? Draw a small area.

(b) **Veins.**

(1) From a stained transverse section of one of the larger veins, make a drawing indicating the number and nature of its tunics. Make a detailed comparison between the structure of the vein and that of the artery.

(2) Compare a longitudinal section with the transverse. Are there any differences to be noted in the appearance of the tunics as cut in the two planes? Are there valves? What is the nature of the latter, their most common location, and whence and how do they originate?

For the variations in the structure of the different veins and the different arteries of the body, see the text-books and the literature on the subject. What can be said, in general, of the walls of the veins below the heart as compared with those above it? Specific structural differences between the vena cava, the mesenteric vein and the jugular? In which groups of veins are valves more common, and in which are they probably absent?

(c) **Capillaries and pre-capillary vessels.**

Place a small sheet of mesentery or pia mater in 1 per cent. silver nitrate for one to twenty-four hours. Then spread flat on the slide, mount in glycerin and expose to diffuse sunlight for half an hour or more. What is the structure of the capillaries? What modifications have taken place in the reduction of the arteries and veins into capillaries? Draw, showing structure and a branching. Abundance and arrangement of muscle? Then draw transverse sections of two or three sizes of these vessels from any of the stained sections showing them. May *arterioles* be distinguished from *venules?*

2. **The heart** (Cor) (human or hog).

(a) **External features.**

(1) The pericardium, the visceral portion (epicardium) and the parietal portion. Where do the two portions become continuous? What fundamental tissues compose the pericardium?

(2) What is the general shape of the heart? Identify right atrium and left atrium with their auriculæ; right ventricle and left ventricle. How do the chambers of the two sides differ in external features? Identify the coronary sulcus (auriculo-ventricular ring) and the interventricular sulcus.

(3) Vessels. Superior and inferior venæ cavæ and the aorta. Relative positions and physical differences? Pulmonary artery

and pulmonary veins. With probe determine with what atrium or ventricle each communicates. Nature of the blood they carry? Identity the superficial coronary vessels.

(b) **Internal features.**

There are several procedures for making the incisions of the heart for study and for autopsies. The following procedure is excellent from the fact that it allows examination of all the important structures and, at the same time, the parts remain connected and easily return to their original positions for purposes of orientation.

(1) Hold heart with right and left side as they are in the body and, with scissors, make the following incisions:

Right Side:

(i) Join the venæ cavæ, cutting from orifice of superior to that of inferior.

(ii) Cut down right border of heart between cusps of tricuspid valve to end of right ventricle (does not reach apex).

(iii) Begin a little above end of the last incision (to save anterior papillary muscle) and pass to and up the interventricular septum to the pulmonary artery.

Left Side:

(i) Join pulmonary veins (4 orifices) and extend cut into left auricle.

(ii) Thence, between bicuspid valve (mitral valve), along left border (middle of the external wall of left ventricle) to apex.

(iii) From termination of last incision, upwards close to interventricular septum, to the aorta, avoiding cutting the semilunar valve. To avoid this it may be necessary to dissect away some of the pulmonary artery from the aorta.

(2) Having completed the incisions, identify the following structures:

(i) Right atrium: sinus venosus, right auricle, septum auricularum, orifice of vena cava with its Eustachian valve, coronary sinus with its orifice, fovea ovalis (with foramen ovalis?), pectinate muscles and crista terminalis.

(ii) Right ventricle: tricuspid valve with anterior, posterior and medial cusps; orifice of pulmonary artery with its valve; papillary muscles, and chordæ tendineæ. Does the muscular wall vary in thickness?

(iii) Left atrium: left auricle, orifices of veins, etc. Which veins? Differences from right atrium?

(iv) Left ventricle: bicuspid valve (mitral valve) with its anterior (aortic) and posterior cusps; semilunar valve with its three cusps and the sinus aortæ (valsalvæ); the coronary arteries, papillary muscles, trabeculæ carnæ, etc.

Make a sketch showing the internal structures of one side of the heart.

(c) **Dissection** of muscular walls. By pulling apart and teasing a heart (fetal pig) which has been macerated in strong nitric acid, verify the following statements:

(1) The heart is composed very largely of muscular tissue which is arranged in layers.

(2) The atria consist of a superficial set of muscle-fibers common to both, and of a deeper set, proper to each.

(3) The ventricles consist of several layers of muscle forming the myocardium. Nearly all of these layers begin in the auriculo-ventricular ring of one ventricle and terminate in the papillary muscles of the other; *i.e.*, those which begin near the outside of one ventricle end near the inside of the other.

(4) The thin superficial muscles removed, the left ventricle is formed of scroll-like, flat bands of muscle, continuous with the muscle bands which cross over in the suptum from the right ventricle. Therefore, taking the layers together, the ventricles consist chiefly of interfitting, scroll-shaped bands of muscle with tendons at each end. Determine the existence and position of a bundle which belongs exclusively to the left ventricle.

What is the position, size, shape and physiological significance of the atrio-ventricular bundle ("Bundle of His")? Does the heart possess skeletal structures?

(d) **Sections.**

(1) From a stained transverse section through the ventricular wall of the heart (human) make a drawing showing its three layers analogous to the tunics of a blood-vessel. What is the nature of the epicardium? The sublayers of the endocardium? Can the myocardium be sub-divided into the component bands above mentioned?

(2) From a longitudinal section through the left atrium and mitral valve, determine the origin of the valve segments. On which side of the segments is fibrous connective tissue most abundant? Origin and arrangement of the muscles of the valve? Draw and indicate.

(3) Blood-supply of the heart. From a section of an injected heart draw a small area showing the arrangement of the intrinsic blood-vessels. What can be said of the abundance of the blood-supply?

B. The Lymphatic System.

1. Lymph-vessels.

(a) Make a careful drawing of a stained transverse section of the *thoracic duct.* Of the vessels previously studied, which does it most resemble in structure?

(b) From a section of the intestine, for example, draw under high power a small area of the *mucosa,* showing *lymph capillaries* and *lymph-spaces.* What can be said as to their lining and the thickness of their walls? What other and larger cavities of the body may be classed as lymph-spaces?

(c) From a preparation in which the lymphatics have been injected, draw a small vessel showing its characteristic contour, thinness of wall, and evidence of frequent valves. Has it an epithelial lining? Differences from blood capillaries?

2. Lymph-glands (lymph nodes).

(a) Fresh. Observe the groups of lymph-glands in the neck, axilla or groin and the scattered lymph-glands in the mesentery of a dog or cat. What can be said as to their color, size and shape? Cut a gland, scrape out some of its tissue, mount in salt solution and examine under high power. What is the shape and general character of the cells?

(b) Make a sketch of an injected and stained sagittal section of one of the medium sized lymph-glands (dog or cat). What is the general shape of the gland? Note the connective tissue capsule with its ingrowths or *trabeculæ* which separate the cortex of the gland into *corticle follicles.* How do the follicles differ from the *medullary substance?* What can be said of the blood-supply and the relation of the blood capillaries to the *lymph sinuses?* Under high power draw an area from the center of a follicle or nodule. How do the cells of the germ center differ from other cells of the follicle? Cell divisions? What is the variety of the connective tissue? Occurrence of blood sinuses?

(c) Lymph-*nodules, solitary* and *agminated,* will be best considered in connection with the alimentary tract.

3. Hemolymph-glands.

(a) What is the distinctive appearance of these bodies in the fresh condition and where are they found in man? Average size as compared with the ordinary lymph-glands?

(b) Draw a stained sagittal section of a hemolymph-gland (sheep or human) showing the structure and arrangement of the capsule, the thickness and extent of the peripheral blood sinus with the reticular connective tissue and the variety of cells contained in it,

and the structure of the medullary portion of the gland. What is the relation of the lymph-vascular system to the blood-vascular system of this gland as compared with those of the ordinary lymph-gland? Compare with descriptions of marrow lymph-glands.

Review bone marrow, considering it as an organ of the lymphatic system. Discuss the lymph-glands as both mechanical and chemical filters interposed in the circulation.

4. **The spleen** (Lien).

(a) Fresh (dog). What is the position, color and shape of the spleen? Where do the vessels enter it? With a sharp scalpel cut out a wedge and examine it in salt solution under the dissecting microscope. Carefully observe both the outer surface and the cut surface, and make small sketches illustrating both. What are the small, lighter spots in the cut surface? Scrape out some of the tissue, mount in salt solution and examine under high power. What different cells can be observed? Carefully note the contents of a large splenic cell. Compare the cells with those of the ordinary lymph-gland.

(b) From a stained vertical section of the spleen, make a detailed drawing of the *spleen lobule*, including the *capsule* of the spleen, the *interlobular trabeculæ*, and the *intralobular trabeculæ*. Compare the *malpighian corpuscle* and the germ center with the follicular nodule of the lymph-gland. How do the cells of the corpuscle differ from those of the *spleen pulp?* What are the *spleen cords?* Note the intralobular venous spaces. Examine, under high power, for the fine framework of reticular connective tissue. In the spleen pulp, look for the various types of leukocytes and for indications of the development of red blood-corpuscles. Draw, under high power, examples of four varieties of the cells contained in the spleen. Nature of the inclusions of some of the large splenic cells and the function of the spleen indicated by them?

(c) Blood-supply. From a section of injected spleen, make a drawing showing the afferent and efferent blood-vessels of a lobule. How does the artery enter the spleen? How is it disposed within the lobule? Look for *ampullæ of Thoma*. How do the veins arise? How does the arterial blood pass into the venous system? What is the relation of the inter- and intralobular veins to the trabeculæ? Number of trabeculæ concerned in each lobule?

C. Glands with Internal Secretion (the ductless glands).

The lymph-glands and the spleen are frequently classed as "ductless glands," though neither in their general architecture nor in their elementary details may they be considered to present any of the features

characteristic of true glandular structure. On the basis of function, also, the definition of "gland with internal secretion" might be strained sufficiently to include them, for not only do both the lymph-glands and the spleen receive and act upon substances from the blood, but they also contribute substances to the blood, modified plasma (lymph), white blood-corpuscles, and, at times, the spleen and marrow contribute red blood-corpuscles. However, upon the basis of their structure, and the fact of their being directly interposed (as nodes) in the lymph channels, both, the lymph-glands especially, are more appropriately studied as intimate parts of the lymphatic system proper. The structures more particularly classed as "glands with internal secretion" are those which follow. They are arranged for study in the order in which they differ in structure from the so-called lymph-glands.

1. The thymus.

(a) From texts and atlases and, if possible, in a human fetus or infant, determine the position, attachments, shape, size, and color of the thymus. Note its very evident lobation and its consistency. Become familiar with its origin and development and with its variable occurrence and persistence at different ages. At what age is it usually largest and at what age is its atrophy usually completed?

(b) From a stained section, sketch a lobule showing its detailed structure, its relation to other lobules, and the nature of the interlobular tissue. What functions may be inferred from its structure and in what does it differ from an ordinary lymph-gland? Under high power draw one of the thymic (Hassall's) corpuscles with its immediate surroundings. Where are these situated and at what age are they most abundant? Their origin and significance?

2. The glomus caroticum (the "intercarotid gland".)

(a) Learn its situation, size and color in man. From a stained section draw a small area under medium magnification, showing its structure. What is its origin and probable function? What structures of the pancreas does it resemble and in what features? Read up the "*cocygeal glands*" and compare.

3. The hypophysis cerebri (pituitary body).

(a) From models and atlases and from a demonstration specimen of the brain itself, determine the position, connections, shape, and orientation of the hypophysis. Its size and color in the fresh? What are the conditions under which it has been found to vary?

(b) Draw under low power an entire stained vertical section passing transversary through the long axis of the body, and thus in the direction of its infundibulum and involving both its posterior and

anterior lobes. Show carefully the difference in structure between the two lobes. Which lobe is the larger? What is the difference in their origin, and therefore, which is directly continuous with the infundibulum? What is the structure of the latter and what is its relation to the tuber cinereum and third ventricle of the cerebrum? Which of the two lobes is most concerned in the enlargement of the hypophysis accompanying acromegaly and gigantism? Look for vesicles (follicles) containing colloid substance. In which border of the lobe containing them are such more usually found? What can be said of the nerves of the two lobes?

(c) Under high power, draw a small area presenting the typical structure of the anterior (glandular) lobe, showing the branched epithelial cords, the arrangement, character, and the varieties of the cells, and the abundance and sinusoidal character of the capillaries. Compare the general structure of this lobe with that of the parathyreoids below.

4. The thyreoid gland.

(a) Determine the position, shape, and lobes of the human thyreoid from models, texts and atlases. How is it attached and with which laryngeal and tracheal cartilages is it most intimately associated? Examine the fresh thyreoid of the dog, noting its color, consistency to touch and its comparative position. Make an incision and note the peculiar character of the exudate. Review the origin of the thyreoid gland and the occurrence of vestiges of its duct. What can be said of the thyreo-glossal duct of man? What phenomena are accepted as resulting from the arrested development, the atrophy and the enlargement of the thyreoid?

(b) From a stained section of a lobe (human), draw one or two vesicles (follicles), showing the nature of their epithelial lining, and the "colloid substance" enclosed, and the intervening connective tissue and blood-vessels. What varieties of epithelial cells are noticeable? What is the general shape of the vesicles? Their variations in size and contents? Cells in the colloid substance? Abundance and peculiar character of the blood-supply? What is the intimate relation between the vesicles and the blood capillaries and lymphatics?

(c) The Parathyreoid "glands." State the position, relation to the thyreoid, variable occurrence, and functional significance of these structures. Difference in color from the thyreoid? Draw an area from a stained section of one of them, showing the branched epithelial cords, the two varieties of cells composing them, and the supporting tissue. Occurrence of vesicles? Is colloid substance ever found? Compare with structure of hypophysis.

5. The suprarenal glands (*Adrenals*).

(a) From models, texts and the human cadaver, determine the normal position of the suprarenals, their relations to the kidneys, their shape, size and their peculiar color in the fresh. Which is usually the larger of the two? Why did the older anatomists call them "suprarenal capsules?" How do those of the dog differ in shape and position from the human? Enumerate the arterial branches supplying the suprarenals. Nature of the nerve-supply?

(b) From a stained section taken vertical to the surface of a suprarenal gland (human, monkey or dog), draw a narrow strip passing from periphery to center, illustrating cortex and medulla, and showing in detail the fibrous capsule (two layers?), the zona glomerulosa, zona fasciculata, and zona reticularis. Note carefully the differences in the structure and staining properties of the cells of the different zones. What is the significance of the occasional isolated clumps of cells situated among the cell-cords of the medulla but having the stain reaction of the zona fasciculata? Origin of the different zones?

(c) Add to the above drawing, opposite each locality, careful drawings under high power of two or three cells of that locality, giving special attention to cell structures and staining differences. Which cells contain oil globules? Give an explanation of the fact that the medullary substance is differentially colored by chromic acid and its salts.

(d) Blood-supply. From a thick section of an injected specimen, make an illustration of the distribution of the capsular, cortical and medullary arteries. Where are the venous capillaries situated? What is the significance of the similarly derived but separate blood-supply of the cortical and medullary substances? What is the nature of the walls of the suprarenal blood-vessels? Explain the arrangement of the cells into cords.

LITERATURE ON THE CIRCULATORY SYSTEM AND GLANDS WITH INTERNAL SECRETION.

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II. THE DIGESTIVE APPARATUS.

(EIGHTH PAPER.)

A. **The Mouth Cavity** (*cavum oris*).

1. The lips.

(a) With a mirror examine the lips and describe the differences to be noticed in the transition of epidermis proper into oral mucous membrane. Is there a sharp line marking the division between the two? Explain the differences in color and evenness of surface.

(b) From a vertical transverse section of the labium inferus make a drawing illustrating the following features: (1) the variations in the epithelium in the transition of epidermis into oral mucous membrane; (2) the variations in the height of the papillæ corii; (3) the relative abundance of the tela subcutanea and the tela submucosa, and the presence of glandulæ sebaceæ and sudoriferæ in the one and glandulæ labiales in the other. What constitutes the tunica propria in the one and the tunica mucosa in the other? (4) vertical muscle-fibers (M. depressor septi) and horizontal muscle-fibers (M. orbicularis oris). Variety of muscle? In the papillæ of the submucosa, look for corpuscles of Krause (corpuscula bulboidea). What can be said of the relative abundance of the tela subcutanea on the back of the hand, of the lip, of the hard palate and of the tongue?

2. The tongue (*lingua*).

(a) With the aid of a mirror and magnifying glass sketch the dorsal surface of the tongue, paying special attention to the following features: (1) the apex and the sulcus medianus linguæ; (2) the tonsillæ linguales and the sulcus terminalis linguæ; (3) the arrangement number, shape and size of the papillæ vallatæ; (4) the size and distribution of the papillæ fungiformes, and (5) the appearance and position of the papillæ folliatæ, papillæ conicæ, and papillæ filiformes.

(b) Hold the tongue up as high as possible, and sketch the following features on its ventral aspect: (1) apex and frenulum linguæ; (2) plicæ fimbriatæ; (3) vanine veins (venæ sublinguales); (4) plicæ sublinguales and carunculæ sublinguales with orifices of (Wharton's) ducts of submaxillary glands, and bulges of the mucous membrane indicating the position of the glandulæ sublinguales.

(c) From vertical sections taken from various regions of the dorsal

surface make drawings illustrating the shape, structure and relative size of five forms of lingual papillæ. How are they formed? What is their relation to the papillæ of the submucosa? Let one of the drawings include such lingual muscles as the section may contain and attach the names of the muscles identified. In which papillæ are situated the specialized corpuscles of the sense of taste? From the papillæ folliatæ of the rabbit make a detailed drawing of a taste-bud (calyculus gustatorius).

3. The soft palate (*palatinum molle*).

(a) With mirror observe the shape, position and facility of movement of the soft palate. Make a sketch showing the uvula palatina, tonsilæ palatinæ, the fossæ supratonsilares, and the arcus glossopalatinus.

(b) Draw a vertical median section of the uvula showing the kind of papillæ, the glandulæ palatinæ and their ducts, the vertical M. uvulæ and oblique fibers of the M. levator veli palatini. Variations of the epithelium of the two sides? Position and variety of the glands?

4. The glands of the mouth (*glandulæ oris*).

(a) *The small glands.* Examine the glandulæ labiales, linguales and palatinæ in the sections studied in 1 (b), 2 (c) and 3 (b). What differences are to be noted as to the size and staining properties of the cells of the various glands? Draw under high power a transverse section of a duct and one or two alveoli from both a mucous and a serous gland, illustrating their differences. Look for crescent cells or "demilunes of Heidenhein." In which glands are they found? What is the difference between an alveolus and an acinus? Nature of the membrana propria about the glands?

(b) *The salivary glands proper.*

(1) Examine a piece of fresh salivary gland under the dissecting microscope. Note color, lobules and alveoli. Sketch a small area of the surface. Now tease a bit of the gland on a slide, mount in water and examine under the compound microscope. Note the connective tissue frame-work and the arrangement of the gland cells. Sketch a single alveolus.

(2) From a section stained in hematoxylin and congo red draw a small lobule of a parotid gland (gl. parotis) showing the connective tissue frame-work, the alveoli, the intralobular and interlobular ducts and including the membrana propria. The distribution, origin and significance of the so-called basket cells? Judging from its staining properties, what is the functional nature of this gland? Under high power, draw one or two

alveoli and a duct in section. Why should the two stain differently? Include an intermediate (intercallary) duct and note the relative size and position of its cells.

(3) Under high power, draw one or two mucous alveoli from the sublingual gland (mucous), or from the submaxillary gland (mixed), illustrating the structure and relative size of the mucous cells and the arrangement and staining properties of the demilunes of Heidenhein (Gianuzzi). Microchemical differences of the latter? Their variations and significance?

(4) From a section of injected salivary gland make a sketch illustrating the blood-supply. What can be said as to its abundance? Arrangement and abundance of capillaries for supply of alveoli?

(5) Read articles cited on the nerve-supply of the salivary glands. Type of axones? Distribution of telodendria to gland cells?

5. The tonsils.

(a) With mirror examine carefully the tonsillæ palatinæ, noting their position and indications of crypts (recessi) in their surfaces and evidence of underlying lymph-follicles. What are the differences in gross appearance between the lingual, palatine, and pharyngeal tonsils?

(b) From a section of the palatine tonsil draw a region containing and surrounding a crypt or recess, paying attention to the modifications of the mucous membrane. What is the tonsil as an organ? Make a high power drawing showing the peculiarity of mucous membrane of the recess and the tissue underlying it. What can be said of the tela submucosa? Invasion of leukocytes?

6. The teeth.

(a) State the number, names, and positions of the teeth. Differences between deciduous and permanent teeth? With a mirror observe evenness of the corona dentis of the incisors and the tuberculæ coronæ dentis of the molars. What is the neck of the tooth (collum dentis)?

(b) Under low power, make a drawing of a ground longitudinal section of one of the incisors, showing the enamel (substantia adamantina) with the appearances which characterize it, the cementum (substantia ossea dentis), the dentin (substantia eburnea), and the pulp cavity (cavum dentis). Is there a foramen apicis dentis? How does the collum dentis differ from the other parts?

(c) Under high power, make a drawing of a segment of the section including the enamel (sub. adamantina), and passing to the pulp cavity. What are the differences between the enamel fibers and the dentinal tubules? What is the nature of the zone dividing the

two? What is the behavior of the tubules upon approaching this zone? What are the lines of Retzius and the lines of Schraeger? Significance of interglobular spaces?

(d) Make a careful drawing of a portion of the cementum (sub. ossea) where it is thickest. In what does it differ from the other parts of the tooth in texture, structure and origin? Significance of Tomes' granular layer?

(e) *Development of the teeth.*

(1) Read carefully the texts and articles cited and fix in mind, in their natural sequence, the various stages in the development of the teeth from the earliest indications to the time of emergence. Make a table showing the average ages of the infant at which the various teeth emerge.

(2) From sections made transverse to the jaws of fetuses of different ages (pig), make sketches illustrating first, the origin of the enamel germ; second, the formation of the dentinal papilla and the dental lamina connecting the germ with the dental furrow. From a vertical section through one of the developing incisors draw under low power showing, third, the differentiation of odontoblasts and adamantoblasts, the formation of the enamel pulp, the outer epithelium of the enamel organ, and the germ of the permanent tooth. Fourth, from a similar section through a later stage, draw the enamel organ alone showing the beginning formation of enamel and dentin. Fifth, under high power draw a few adamantoblasts and odontoblasts showing their differences, relative positions and products.

What is the origin and significance of the membrana præformitiva? Relative shape, size and arrangement of adamantoblasts and adontoblasts? How is the germ of the permanent tooth connected with the enamel organ of the deciduous tooth and with the oral epithelium? Nature of the dental ledge? Differences between enamel pulp and tissue contained in dentinal papilla (dental pulp)? What occupies the dentinal tubules?

B. The Pharynx and Esophagus.

1. *Fresh.* Note the extent and variations of the tube in position. Remove a small piece of the fresh esophagus and tease it in salt solution under the dissecting microscope, identifying the following coats: epithelium, tela submucosa, tunica muscularis with its stratum circulare and stratum longitudinale, and the tunica adventitia.

2. Draw a segment of a stained transverse section taken through the pharynx, and then of one taken through the lower third of the esophagus. First study the sections carefully, noting all dif-

ferences between the two. What is the nature of the epithelium? At which level are glands most abundant? What is the nature of the glands and how do their secretions reach the surface? Where and how does the muscularis (lamina) mucosæ begin? What differences are to be noted in the outer muscle layers (strata) of the two sections? Look for superficial esophageal glands. Where and how are these situated? Show their structure. Significance?

C. **The Stomach** (*ventriculus, gaster*).

1. Clip out a small block of the wall of a fresh stomach (dog) and place it in normal salt under a dissecting lens.

(a) With a pipette wash off the débris from the glandular surface and observe the epithelium closely. Make a careful sketch of a small area showing the orifices of gastric crypts?

(b) Now tease the specimen, identifying the following tunics: epithelium, lamina propria mucosæ, muscularis mucosæ, tela submucosa, tunica muscularis consisting of stratum circulare and stratum longitudinale, and finally, tunica serosa. Are fibræ obliquæ to be noted? In what region of the stomach are they present?

2. From a section (stained) passing vertically through the junction of the esophagus and stomach (cardia), make a drawing under low power showing the transition of the epithelium of the one into that of the other and the change in the character of the glands.

3. **Cardiac Glands.**—Make a small drawing showing in detail the structure of these glands. Parietal cells? Goblet cells? Significance of the glands? Extent of area occupied?

4. **Fundus Glands.**—From a section taken vertically through the wall of the fundus ventriculi, make a drawing one or two gastric crypts in width and involving all the tunics, including the tunica serosa. How many glands open into a single crypt? What is the relative abundance, position and staining qualities of the parietal or oxyntic (delomorphous) cells as compared with the chief or peptic cells? What is the extent of the mucosa (lamina propria) and the relative thickness of the tela submucosa? What constitutes the mucous membrane? Under high power draw a small part of one of the longitudinally cut glands, showing in detail the relation and structure of the parietal and chief cells, and their relation to the basement membrane. In what part of the cells are the nuclei situated? Are granule cells of Paneth present? To what tunic belongs the tissue just under the basement membrane?

5. Draw a small segment of a fundus gland prepared by the Golgi method, showing the lumen of the gland and the secretory duct and secretory capillaries of two or three parietal cells. Do all the cells have a duct? What is indicated by the occurrence of a duct and capillaries? Are the adelomorphous cells affected by the silver? Why?

6. **Pyloric Glands.**—From a section taken transversely through the wall of the pars pylorica, make a drawing including the mucous membrane only, and showing the form and nature of the glands (glandulæ pyloricæ). Closely observe and enumerate all the features in which this region differs from that of the fundus. Evidences that one of the chief functions of the pyloric glands is mucous secretion?

D. **The Intestine.**

1. **Duodenum.**—Under low power sketch a section passing longitudinally through the pylorus and involving a portion of the pars pylorica ventriculi on the one side, and the duodenum on the other. Give special attention to the zone of transition of the mucous membrane, the abundance and position of Brunner's glands (glandulæ duodenales) and the M. sphincter pylori. What constitute the differences in the epithelium? What is the functional nature of Brunner's glands? What muscle stratum enters chiefly into the formation of the sphincter pylori? Occurrence and origin of muscularis mucosæ?

2. **Small intestine** (*intestinum tenue*).

(a) From a transverse section either of jejunum or illeum, make a drawing showing one or two villi and the adjacent crypts of Lieberkuehn, and including all the tunics. What is the relation of the stratum proprium (lamina propria) to the villus? Muscularis mucosæ? What of the staining qualities, position, shape and distribution of the goblet cells? Does the section involve a Pyer's patch? What are these and where situated? Granule cells of Paneth?

(b) Under high power draw a few of the epithelial cells of a villus including a goblet cell and a portion of the villus axis. Granule cells of Paneth, their position and significance? Is there a striated cuticular zone? Basement membrane? What is the structure of the goblet cell and how does it differ from the chief cell? What are the differences between the mucous membrane of the jejunum and illeum?

3. **Large intestine** (*intestinum crassum*).

(a) Make a drawing from a transverse section of the large intestine including only the mucous membrane and showing one or two of the glands of Lieberkuehn. Let this or a separate drawing show a solitary lymph follicle. Make a detailed comparison between the arrangement of the epithelium here and that of the small intestine. How do the goblet cells differ? What is the arrangement of the epithelium lying over the "solitary lymph-gland?" How does the tunica muscularis differ from that of the small intestine and how are the tænia and haustra coli produced?

4. **Appendix** (*processus vermiformis*).

(a) Draw showing a portion of a transverse section from a normal specimen. Note and show the nature and condition of the epithelium, the relative abundance of the lymphatic follicles and the modifications of the tunica muscularis as compared with the small intestine. Muscularis mucosæ? Size of the lumen? Significance of the structure of the appendix? Its origin?

E. Rectum and Anus.

1. Sketch a longitudinal section showing the transition of the epithelium of the rectum into that of the anus. Type and arrangement of the glands? In what does the rectum differ from the large intestine? Modifications of the musculature of the anus from that of the rectum? Striated muscle? Nature of anal glands proper?

F. Blood-supply of the Digestive Canal.

1. From a section of an injected stomach or intestine, sketch a small area showing the blood-supply as distributed to the various tunics. Note that the vessels, entering by way of the mesenteric attachment, form (a) the intermuscular plexus and (b) the plexus of Heller. The latter supplies the mucous membrane. What can be said of the relative blood-supply of the different tunics?
What is the general arrangement of the lymph-vessels of the digestive tract?

G. Nerve-supply of the Digestive Canal.

1. Examine all sections of the canal supplied, (a) between the strata of the tunica muscularis for nerve-cells belonging to Auerbach's plexus (plexus myentericus) and (b) in the tela submucosa for cells belonging to Meissner's plexus (*plexus submucosa*). Sketch a group of cells showing its surroundings.
2. Tease apart the above tunics of a piece of a stomach or intestine, which has been stained with gold chlorid; mount bits of both the

submucosa and tunica muscularis in glycerin and examine for the above named plexuses. Sketch showing ganglia and the arrangement of the nerve fasciculi forming the plexuses. What is the shape of the ganglion cells? Significance of the plexuses? Nature and origin of cerebrospinal axones supplying the digestive canal?

H. The Liver (*hepar*).

1. *The fresh specimen.*

(a) With the organ in position in the body, note its lobes, right and left; its relations to the diaphragm, duodenum, stomach, kidneys and colon. What is the color of the organ? How is the vena cava inferior attached to the organ? Note the entrance of vena portæ. From what visceral veins is it derived?

(b) Remove the liver from the subject and identify the following structures: lobus dexter, lobus sinister, and lobus caudatus; vena portæ with right and left divisions; vena cava inferior with venæ hepaticæ dextra and sinistra; arteria hepatica with right and left divisions; ductus hepaticus, ductus cysticus, ductus choledochus (common bile duct), and vesica fellea (gall cyst). Trace the ductus choledochus. Into what does it open? Open the gall cyst, and with lens note plicæ tunicæ mucosæ. Split the cystic duct and observe the valvula spiralis. What is the nature of its formation? Note lig. teres, and on anterior surface, lig. falciforme hepatis and lig. coronarium hepatis.

(c) With dissecting lens, examine the surface of the fresh liver, noting its general appearance and the indication of lobules. Remove a wedge of the organ and tease in salt solution. What is the approximate size of the granules and what are they? What can be said of the abundance of the frame-work?

2. Sections.

(a) Injected (dog or cat). Under low power draw a single lobule or hepatic unit in transverse section, showing its shape and relation to adjacent lobules, the venæ interlobulares and the anastomosing system of intralobular capillaries converging into the intralobular vein or vena centralis. Note anastomoses of capillaries in the third dimension. Draw in some of the liver cells. Look for a lobule cut longitudinally and thus showing the vena sublobularis. What is the relation of the latter to the vena centralis and to the hepatic vein? What is the course of the blood borne by the hepatic artery? Note the interlobular capsulæ fibrosæ, or capsules of Glisson, and their surroundings and identify the three larger vessels contained. What are "portal canals?"

(b) Stained sections (human and pig). Under high power draw (1) a quadrant of a lobule including the vena centralis and the portion of the interlobular frame-work and vessels involved. What is the arrangement and thickness of the columns of liver cells (hepatic cords) and what is their relation to the capillaries? The relation of the individual cell to the capillary wall? Note occasional "Stellate cells of Kupffer" and look for evidences that they serve as a partial endothelial lining of the capillaries and that they consist of a syncytium of epithelial cells. (2) With oil immersion, draw two or three cells showing their internal structure in detail. Do they possess cell membranes? Relation to bile capillaries? Look for secretory channels (bile canaliculi). Nature of the granules? Evidence of fat globules? Pigment? In what functional stage are the cells? What can be said of cell division in the liver? (3) Draw a capsule of Glisson. What structural differences distinguish the three types of larger vessels it contains? How do the larger bile ducts differ from the smaller? In what does the human liver differ from that of the hog?

(c) Golgi preparation. Draw a quadrant of a lobule (hepatic unit) showing the intralobular net-work of bile capillaries as differentiated by silver, choosing an area which shows the capillaries continuous into an interlobular bile duct. Look for bile canaliculi. Explain their occurrence.

(d) Frame-work of the liver. Either digested preparation or one specially stained for the frame-work. Draw illustrating the abundance, quality, and arrangement of the connective-tissue frame-work of a lobule and the adjacent interlobular tissue. What variety of fibrous connective tissue predominates? What is the relation of the fibers to the liver cells and to the course and structure of the intralobular blood capillaries. Since no typical connective-tissue "cells" are to be observed within the lobules in any of the preparations, whence arises the intralobular frame-work?

Distinguish between the hepatic unit (blood-vascular unit) and the portal unit (secretory unit) and, on the basis of development and function, show which of these units can with more reason be considered the unit of structure of the liver.

3. The gall-bladder (*vesica fellea*).

(a) Macroscopic. From a fresh specimen (ox or hog) or from a gall-bladder which has been fixed and dried distended (human), identify the ductus choledochus, ductus hepaticus, ductus cysticus, and the shape and relative position of the vesica fellea. What is the direction of the connection with the duodenum? Beginning with the collum vesicæ, note indications of the spiral (Heisterian) valve.

Open the fresh gall-bladder and note the numerous, irregular polygonal depressions produced by the continuous system of permanent corrugations or folds of the mucosa.

(b) Under high power draw a portion of a stained vertical section of the wall of the gall-bladder, showing all of the tunics. Evidence that the corrugations are permanent? Type of the epithelium? Goblet cells? Indications of fat globules? Lymphatic nodes and indications of lymphatic vessels? Into how many and what strata may the muscle of the fibro-muscular tunic be arranged? What is the extent and origin of the tunica serosa? Variations in the wall in different regions? Mucous glands?

I. Pancreas.

1. *Macroscopic.*

(a) Observe the organ in position (dog or cat). What is its relation to the stomach and duodenum? Color? Identify caput, cauda and ductus pancreaticus. What part of the duodenum does the duct enter? Note that the gland is divided into lobes held together by ingrowths of its capsule (membrana propria). Make a sketch illustrating the surface appearance of a lobe.

(b) Tease a bit in salt solution, mount and examine under high power. What is the shape and arrangement of the gland cells? Note that the shape of the alveolus suggests the name "acinus."

2. *Stained sections.*

(a) Under high power draw a transverse section of a duct and one or two alveoli showing "centro-acinar" cells and an intermediate (intercallary) duct. How do the ducts differ from the alveoli? Do the cells of the alveoli have an inner granular zone? Explain it? Position of the nuclei? Note that the larger ducts are lined either by pseudo-stratified or stratified columnar epithelium. Is there any muscle in their walls?

(b) Sketch one of the intralobular cell masses or "*islands of Langerhans*," including the tissue immediately surrounding it. Describe it. What varieties of cells compose it? What is its origin and significance and what is its shape as shown by reconstruction? In what part of the pancreas are these cell masses most abundant? What gland previously studied does the pancreas most resemble in structure and function? State five particulars in which the two differ.

(c) Frame-work. From a thick section of digested pancreas stained with fuchsin or analin blue, draw under low power an area involving an island of Langerhans. What is the relation of the connective tissue fibres to the alveolar cells and to the ducts? What is the significance of the especially abundant frame-work of the islands?

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III. THE RESPIRATORY APPARATUS.

(NINTH PAPER.)

A. Appearance in Fresh.

1. Saw transversely through the nares (dog). Note the paired cavity lined with a thick mucous membrane and the means by which the area of this membrane is increased. Are glands observable? With what cavities is the cavum nasi continuous?
2. Examine the larynx, trachea and lungs in position in the body (dog or human), identifying the single and paired cartilages of the larynx and their relation to the hyoid bone, and note the glandula thyreoidea and thymus and the laryngeal muscles; the trachea, its relation to the esophagus, and the number and shape of its semilunar cartilages. Where are the free ends of the tracheal cartilages? Where are the glandulæ tracheæ? Note the right and left bronchus to right and left lung. Right and left pulmonary arteries and veins. Whence are the bronchial arteries derived?
3. Identify the lobes of the lung. With dissecting lens observe lobules and visceral pleura. What is the nature of the latter?

B. Prepared Specimens.

1. Cavum nasi.

(a) From a longitudinal section through the vestibular region make a drawing showing the transition of the vestibular epithelium into that of the regio respiratoria. Note the change in the type of the glands. Occurrence and function of hairs?

(b) From a stained section of the epithelium taken from the posterior portion of the nasal cavities, make a drawing illustrating the character of the epithelium and showing the structures present in in the mucosa. What can be said of the nature and abundance of the glands? Goblet cells in epithelium? Blood-supply? How does the epithelium here differ from that in the nasus externus? In what details does it differ from that of the olfactory region?

2. Larynx.—Longitudinal section passing transversely through the vocal cords (ligamenta vocalia). Draw under low power showing position and shape of false and true vocal cords, their structure and the ventricle of the larynx between them. What is the nature of the epithelium upon the vocal cords? What other structures of the larynx are similarly covered? Where are the glands most abundant? Lymphoid tissue? Which cartilages are represented in the section?

3. **Trachea and Bronchi.**—Draw a segment of a transverse section of the trachea including the ends of a semilunar cartilage and showing the annular ligament connecting them and the band of transversely arranged smooth muscle. Note especially the character of the epithelium, the basement membrane (membrana propria), the layer of compact and circularly disposed elastic tissue and the looser fibrous tissue next the perichondrium which contains the glandulæ tracheæ. Goblet cells? Where is lymphoid tissue most abundant? Where are the larger mucous glands? Compare a transverse section of a bronchus with that of the trachea and enumerate the differences to be noted.

4. **The lung** (*pulmo*).

(a) From a stained section of the lung draw a transverse section of a small bronchial ramus, of a bronchiole, and of a respiratory bronchiole. Give special attention to the variations in the epithelium as compared with that of the trachea and bronchus, and the variations in the occurrence of glands, cartilage and muscular tissue. What can be said of the disposition and relative abundance of the latter? What characterizes "respiratory epithelium?" Note the abundant supply of arteries, veins and capillaries.

(b) From a thick section of a lung (rat or cat) into which, through the trachea, 1 per cent. silver nitrate has been injected, make a drawing of a pulmonary alveolus continuous into an alveolar duct and respiratory bronchiole, showing the nature, shape and variations of the epithelial cells lining them. What is the relative size, number and position of the nucleated and "non-nucleated" cells? Look for interalveolar passages between adjacent alveoli.

(c) From a Wood's metal corrosion preparation, break out a twig including a bronchiolus, respiratory bronchiole, alveolar ducts, infundibula and pulmonary alveoli. Draw under low power showing three dimensions? What is the shape and nature of the surfaces of the alveoli? Note depressions produced by connective tissue fibers supporting the alveoli.

(d) From a section stained with orcein, sketch a longitudinally cut alveolar duct with its alveoli showing their elastic tissue frame-work. Shape of alveoli? How are the arteries of the section characterized?

(e) From a thick section of an injected specimen, sketch the capillary system of an alveolus. Does it communicate with that of neighboring alveoli? What is the relative distribution of the arterial and venous capillaries about the alveolus?

(f) From the drawings made in b, c, d, and e, reconstruct, in colors, half an alveolus on an enlarged scale, showing from within, the structures in position.

(g) From any section showing it, make a sketch of the visceral pleura, illustrating the nature of the layers of which it is composed.

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Nose, Larynx, Trachea.

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V. THE URINO-GENITAL SYSTEM.

(TENTH PAPER.)

A. The Urinary Apparatus.

1. *Macroscopic appearances.*

(a) Observe the organs in position in the body (human or dog) and identify the following:

(1) The kidneys (renes), their position, shape, color and surfaces. The hilus renalis and its relation to the A. renalis and V. renalis arising from the aorta abdominalis and vena cava inferior. Whence is the nerve supply derived?

(2) The ureters. How do they differ from the arteries and veins in size and appearance, and in their connection with the kidneys?

(3) The bladder (vesica urinaria). On which surface do the ureters open into it? Note lig. umbilicale medium. To what is it attached? Urethra and prostata, where situated? Open the bladder and note plicæ mucosæ, orifices of the ureters and orifice of the urethra?

(b) With razor divide a kidney along its median longitudinal plane and note tunica fibrosa, substantia corticalis, substantia medullaris and medullary rays. Observe the ureter expanded into the pelvis renales with its calyces majores and minores. What is the relation of the columnæ renales to the general supporting tissue of the structures in the hilus? What is their relation to the pyramids and calyces? What is the relation of the pelvis to the hilus? Make a sketch of the section. What can be said of the general distribution of the blood-vessels? Note from the investigations cited that the larger blood-vessels (arteriæ propræ renales) are separated into a smaller ventral and a larger dorsal set.

2. *Microscopic structure of* **the kidneys.**

(a) Sagittal section of injected specimen. Under low power draw a segment passing from hilus to tunica fibrosa, showing the following:

(1) Interlobar and arcuate arteries and veins. Where are they situated? Whence are they derived? In what direction do they run?

(2) The capillary system of the substantia medullaris. Whence are the capillaries derived and what is their general direction? What is the nature of the structure between them? Note arteriole rectæ.

(3) Give special attention to the capillary system of the substantia corticalis opening into the arteriæ and venæ arcuatæ. Note arteriæ and venæ interlobulares. Arising from the interlobular arteries observe twigs (vasa afferentia) which break up to form the renal glomeruli. Arising from the glomeruli are the vasa efferentia which break up into finer capillaries and, as arteriolæ rectæ, those nearest the medulla contribute to the capillary system of the medulla as well as the cortex. Note the capsula glomeruli (Bowman's capsule). What is its nature? With what is it continuous? What constitutes the corpuscula renis (Malpighian corpuscle)? Are arteriole rectæ spuriæ to be observed? Are there arteries entering the kidney at other regions than the hilus? If so, do these also form glomeruli? Enumerate the divisions of the blood-supply of a renal lobule. Of what are the columnæ renales (Bertini) composed?

(b) Examine under dissecting lens sagittally cut pieces of a kidney of a mammal which have macerated twenty-four hours in 30 per cent. hydrochloric acid and washed in running water one hour. Choose pieces favorably cut, place in a drop of glycerin on the slide and very carefully isolate the uriniferous tubules by teasing. Cover and continue the isolation by gently tapping the cover-glass. Identify the different portions of the tubules to be seen under low power, noting especially the form and extent of the glomeruli, the convoluted tubules, the loops of Henle and the relation of the collecting tubules to the papillary ducts.

(c) From a stained section of a human kidney passing from a papilla to the tunica fibrosa, under high power draw small areas showing the character of the uriniferous tubules in the following localities:

(1) A small area of the substantia corticales illustrating a Malpighian corpuscle with glomerulus, capsula glomeruli and the beginning of the uriniferous tubule, the convoluted character of the tubules in this locality and the varying character of their epithelium. How does the epithelium of the capsule differ from that of the tubule? What can be said of the supporting tissue?

(2) A small area from the apex of the papilla, projecting into the calyx, showing the joining of the collecting tubules to form the ductus papillares (ducts of Bellini). How does the epithelium here differ from that of the ducts above? Does the loop of Henle ever extend beyond the arcuate vessels into the medulla?

(d) From the various studies made, reconstruct a renal lobule showing the blood-vessels (arterial and venous) in their relation to the uriniferous tubules and the course of a tubule from the corpusculum renalis, showing the capsule, proximal convoluted portion (tubulus contortus), the ascending and descending limbs of the loop of

Henle (tubuli recti), the distal convoluted portion continuous into the collecting tubules, and the papillary duct.

Read carefully the descriptions of the variations in the epithelial lining of the tubules, noting what these variations signify.

(e) Make a small sketch showing the relative thickness and nature of the tunica fibrosa of the kidney. Does it contain muscular tissue?

3. The ureters.

Make a drawing of a transverse section of the pelvic part of a human ureter showing in detail the character of its epithelial lining, the folds of the mucosa and the inter-relation of the three strata of the tunica muscularis. Examine the pelvis of the kidney (sections above) for the transition of the epithelium of the papillary ducts into the variety found in the ureter. In what does the upper or abdominal part of the ureter differ from the pelvic part? Explain the occurrence or absence of a tunica serosa. Are there glands of the ureters?

4. The bladder (*vesica urinaria*).

(a) *Fresh, macroscopic.* Review the position of the bladder in the pelvic cavity, the structures adjacent to it, the locality and attachment of the medial umbilical ligament (urachus), the localities of entrance of the ureters and exit of the urethra, and the color and texture of the organ. Inflate a bladder by way of the urethra, noting its remarkable capability of distention. What constitutes the trigonum vesicæ? Open the bladder and note evidence of strikingly loose tunica propria allowing numerous folds of the mucosa which are wholly obliterated by distention of the organ. What is the origin of the bladder and urethra? Explain the attachment of the ureters to it.

(b) *Microscopic.* Compare a stained vertical section taken from the body of the bladder with one taken from the trigonum (fundus) near the urethral orifice. Draw a segment from either section, showing the nature of the epithelium and the various strata, including the tunica serosa. How many layers of cells in the epithelium? Relative size, shape and number of nuclei of the layer of superficial cells? Actual shape of the cells of the deeper layers? How many muscle strata and why are they confused? Why are the three strata of the tunica muscularis more definitely arranged in the trigonum than in the general body of the bladder? Relative abundance of connective tissue? In what locality only are glands of the bladder to be found? Nature of their secretion? Explain the fact that the epithelium of the bladder when distended consists of fewer layers of cells and of cells of very different shape as compared with that of the contracted condition. Look for lymph

nodules in the sections. What can be said of the nerve-supply of the bladder? Look for cells of small sympathetic ganglia in its wall. Abundance of the blood-supply? Where are the larger vessels situated?

5. The urethra (*female*).

The male urethra will be studied with the male genital organs. Under low power draw a transverse section showing the folded nature of the mucosa, the scant supply of glands, the peculiar character and arrangement of the strata of the tunica muscularis, and the thick tela submucosa pervaded by numerous blood-vessels, many of which are sinusoidal venous channels giving resemblance to the erectile tissue comprising the corpus cavernosum urethræ of the male. How does the epithelium of the external (vaginal) orifice differ from that of other parts? Which of the muscle strata is concerned and what variety of muscle predominates in the formation of the M. sphincter urethræ?

B. **The Male Genital Apparatus** (*organa genitalia virilia*).

1. *Macroscopic relations.*

(a) Study the arrangement of the component parts of the human genital apparatus in position either from atlases or in the subject in the dissecting room, noting the relations to each other of the following: scrotum; tunica dartos; M. cremaster; tunica vaginalis communis; plexus pampiniformis; tunica vaginalis propria; testis with its tunica albuginea; epididymis with caput, corpus and cauda; ductus deferens with its ampulla, vesicula seminalis and ductus ejaculatorius; prostate gland, and penis with its glans, crus and bulbus urethræ. From dissections, or illustrations, trace ductus ejaculatorius through prostata into urethra, noting colliculus seminalis and utriculus prostaticus. Of the urethra, identify pars prostatica, pars membranacea, pars cavernosa and fossa navicularis. Note the relation of the glandula bulbourethralis (Cowper's gland) to the corpus cavernosum urethræ (corpus spongiosum), and note the arrangement and consistency of the corpora cavernosa penis.

(b) With razor split the testis and epididymis (human or dog) sagitally and under dissecting lens sketch the surface exposed showing the following: tunica albuginea; mediastinum (body of Highmore); the septa dividing the testis into lobules; the tubular structure of the lobules; the rete testis and the epididymis. The ductuli efferentes arise from the rete and join the single but greatly contorted ductus epididymis which is continuous into the ductus deferens at its emergence from the cauda of the epididymis. From the sections to be used in 3 and 4 below, note the abundance, variety and arrangement of the supporting tissue of the parts.

2. Spermatosomes (*spermatozoa*).

(a) Fresh seminal fluid may be obtained most favorable for study (less diluted and containing less mucus) from the epididymis of the dog, the spermatozomes of which animal closely resemble the human. With scissors the fresh epididymis should be snipped into bits into a watch glass of normal salt solution. Mount a drop of the salt solution and examine under high power. Note that the motility of the organisms increases after the first ten minutes in the salt solution and that the movement of those with straight tail-pieces (mature) is more vigorous than that of those bent at the junction of the middle-piece and tail-piece. Some of those bent at first become straight after a few minutes.

(b) At the time the above material is obtained, make a cover-glass, smear preparation of the seminal fluid, fix one hour in Van Gehuchten's fluid, wash ten minutes, first in 95 per cent. and then in 70 per cent. alcohol, stain with hematoxylin and eosin, dehydrate, clear and mount in balsam. Draw one or two spermatosomes under high power, identifying the four parts. Is there a head cap or lance? Undulating membrane? Compare with human spermatosome.

3. Spermatogenesis.—Study the process from thin, stained sections involving lobules of human testis and that of the rat. From the latter draw segments of tubuli contorti seminiferi showing four or five stages of spermatogenesis, including in each drawing sustentacular cells, spermatogonia, spermatocytes, spermatids and further developed spermatosomes. In which cells are evidences of Karyokinesis? On a larger scale, illustrate the internal morphology of a spermatid. State the disposition of its constituent elements in the formation of the mature spermatosome. In drawing the segments of the tubules, include the membrana propria and some of the interstitial connective tissue.

4. Under high power, draw a few of the *interstitial cells of Leydig* from sections of the human testis. Varieties of the cells? Their behavior during the growth and functioning of the gland? Their significance?

5. From sections involving the human mediastinum testis, epididymis and ductus deferens, draw and compare transverse sections of the different ducts, especially noting changes in the epithelium of the *tubuli recti*, *rete*, *ductus efferens*, *ductus epedidymis* and *ductus deferens*. Where do the cilia begin? The three strata of muscle? Compare ductus deferens with ureter. Also make a small drawing illustrating the structure of the vesicula seminalis and compare its structure with that of the ampulla ductus deferentis. Cilia? Variations in tunica muscularis?

6. Prostata.—Sketch a segment of the gland (human) from a section transverse to the urethra, showing the dense muscular and connective

tissue investments and the radiating systems of compound branched alveoli forming the lobules. What is the shape of the lobules? Chemical nature of secretion? How does the succus prostaticus pass into the urethra? Significance of the utriculus prostaticus? What variety of epithelium lines the prostatic portion of the urethra? What is the relation of the muscle of the prostate to the M. sphincter urethræ? In dilated portions of the ducts and alveoli of the gland, look for colloid concretions (prostatic stones). What is the origin and pathological significance of these? What of the abundance of the blood-supply of the prostate and the nature of its nerve-supply?

7. Penis and urethra.

(a) Sketch a transverse section through the *cavernous region* and a longitudinal section involving the *glans penis* (human or monkey). Especially note the corpora cavernosa penis with their tunica albuginea, their septum penis and the character of their structure; the urethra and the character of its epithelium and glands; the corpus cavernosum urethræ (corpus spongiosum) with its tunica albuginea, the distribution of its muscle, its arteries, and the abundance and structure of its cavernous sinuses; the fascia penis with its nerve trunks; the Aa. and V. dorsales and A. profunda, and the integument. Why are the corpora cavernosa so named? From which of them is the glans penis derived? Divide the structure of the corpus cavernosum urethræ into tunica propria mucosæ, tela submucosa and tunica muscularis. How and why does the epithelium of the navicular portion of the urethra (glans) differ from that of the other regions? How many varieties of glands are to be found in the section of the glans penis? Look for corpusculated nerve terminations, especially in the glans. Sections of nerve twigs in the corpora cavernosa? Under high power, draw a small area, illustrating the detailed structure of the corpora cavernosa.

(b) Draw a segment of a section of a *bulbourethral* (Cowper's) *gland* showing the character of the capsule, the secretory alveoli and ducts. Note that the larger of the latter have muscle in their tunics. Look for and determine the significance of both striated and smooth muscle-fibers in the periphery of the section. What is the appearance and chemical nature of the secretion of the gland?

C. The Female Genital Apparatus (*organa genitalia muliebria*).

1. *Macroscopic.*

(a) From atlases or in their position in the body (human or dog), identify the following structures: ovarium with its free margin and ligamentum proprium; tuba uterina (Fallopian tube) with its

fimbria, infundibulum, ampulla, and isthmus; uterus with its fundus, cervix, and external orifice (posterior and inferior labia). Note the relation of the latter to the vagina. How does the uterus of the dog differ from the human?

(b) With razor, divide the ovarium (dog or hog) and, with the aid of dissecting lens, note tunica albuginea and stroma ovarii; corpora lutea and folliculi vesiculosi (Graafian follicles).

(c) From a demonstration specimen of the human uterus divided longitudinally, identify ostea of tubæ uterinæ, cavum uteri, canalis cervicalis, with plicæ palmatæ, and the labia of the orifice. What can be said of the walls of the uterus? Sketch.

2. *Microscopic structure.*

(a) **The ovarium** (ovary).

(1) From a stained section of an ovary of a very young animal or fetus, draw under low power a small area showing germinal epithelium, primordial ova (sexual cells), follicles and stroma. Whence are the ova derived? Show "egg tubes" or "egg nests." Is there any muscular tissue in the section?

(2) *Ovogenesis.*—From stained sections of adult ovaries, make drawings under high power showing the following developmental stages of the follicles:

(i) Primary follicle or oocyte of the first order (folliculus oophori primarii) showing oocyte enclosed by a single layer of flattened or cubical follicular cells and surrounded by loosely arranged stroma ovarii.

(ii) A follicle showing the beginning of the zone of growth, *i. e.* a larger ovum (oocyte) containing yolk granules (deutoplasm) and a germinative vesicle (enlarged nucleus) and enclosed by a zona pellucida surrounded by high cylindrical, radiating follicular cells with the immediately surrounding stroma compressed into a theca folliculi.

(iii) A more advanced stage showing further enlarged ovum with a more distinct zona pellucida surrounded by a solid mass of stratified, radiating follicular cells forming the corona radiata, and a further developed theca folliculi. What is the "germinal spot?"

(iv) A greatly enlarged follicle showing the ovum projecting into an antrum folliculi but still surrounded by a cumulus of the corona radiata (discus proligerus) and attached to the membrana granulosa, or remains of the corona radiata, adhering to the theca. Note now, two tunics of the theca. What is the nature and the origin of the substance which fills the antrum?

Derivation of the zona pellucida? How are oocytes of the second order produced, and what further changes must occur before the maturation of the ovum is complete? Give a mechanical explanation of the extrusion of the ovum. Compare the development and maturation of the ovum with that of the spermatosome.

(3) Study a corpus luteum in section. What is its relation to the ruptured follicle and the corpus hemorrhagicum? Whence are the structures composing it derived? Draw a few lutein cells with the supporting tissue about them, showing their structure in detail. What is the process by which the corpus luteum disappears and what is the origin, structure and fate of the corpus albicans?

(b) **Tuba uterina** (Fallopian tube).

Study and compare a transverse section from the isthmus with one passing through the ampulla. Why is the tunica serosa incomplete? Note evidence that the extensive, compound foldings of the mucosa of the ampulla are permanent folds while the fewer and simpler folds in the isthmus are not necessarily permanent. The tunica propria is vascular and extends into the folds in all regions. In what tunic are the larger blood-vessels situated? In what region does the tunica muscularis consist of three strata and what are the directions of these? Which of these strata persists throughout the tube and thus is present in the infundibulum? Can the innermost stratum be considered a muscularis mucosæ? Note the variety of the epithelium in both sections. Are all the cells ciliated? Does the tube possess glands in any region? Make a careful drawing of the section of the isthmus. Compare it with the ductus deferens and with the ureter.

(c) **The uterus** (non-pregnant).

Draw a narrow strip through the wall from a section through either the body or the cervix of the uterus. Note the great thickness and vascularity of the mucosa and numerous outfoldings of its epithelium forming the so-called uterine glands. What are the reasons for not considering the majority of these foldings as true glands? In the cervix look for evidence of mucus producing cells in the epithelium of the folds. Where are these true (cervical) glands most active? Explain the variable occurrence of cilia. How many strata comprise the tunica muscularis and in what region are these strata most distinct? Abundance of elastic tissue? State the changes in the entire wall which occur during pregnancy.

(d) **The vagina.**

Draw a strip through a transverse section of the middle portion of the vagina, showing its thick epithelium thrown into coarse rugæ and resting upon papillæ occupied by a delicate tunica propria, and including the muscular and cavernous tissues of its wall. Glands are absent in this region. What is the character of the major and minor vestibular glands? To what gland in the male do the major vestibular (Bartholin's) glands correspond? Look for evidences of the ganglionated nerve plexuses in the fibrous tunic.

(e) **The Mammary Gland** (*Mamma*).—One of the glands of the skin.

(1) From a section passing vertically through the mammilla (nipple) and involving the structures beneath, make a drawing showing general arrangement of component tissues, especially the ductus lactiferi (mammary ducts), sinus lactiferi, lobi and lobuli mammæ, and finally, the alveoli. In what does the epidermis covering the mammilla differ from that of other regions? What are the glandulæ alveolares (Montgomery's glands)? Explain the presence and position of muscular tissue in the corium. Note the abundance of paniculus adiposus.

(2) Under high power, sketch one or two alveoli from a section of any mammillian mammary gland in the process of lactation. Show in detail the changes which the gland cells undergo in the production of milk. Look for colostrum corpuscles. During what stage of lactation are they most abundant? Explain their occurrence. Draw one or two separately. Read up the structure of mammary glands of the male.

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THE CENTRAL NERVOUS SYSTEM.

(Portions of the first part of this chapter are modifications of certain paragraphs in Chapter XX of Hardesty's *Neurological Technic*, The University of Chicago Press, 1902).

(ELEVENTH PAPER.)

I. THE SPINAL CORD (medulla spinalis).

A. Macroscopic Study.

1. **Peripheral Connections.**—Fetal pig of 25 to 30 centimeters.

(a) Eviscerate the fetus through a median ventral incision and wash out the body cavity. Note the spinal nerves arranged at intervals (segments) and passing from the dorsal mid-line (axis) of the body. Carefully observe the sympathetic trunk with the ganglia of this trunk, the gangliated cord, extending along either side of the vertebral column. By gently dissecting, trace rami communicantes from the ganglia of the sympathetic trunk into the body wall to connect with the trunks of the spinal nerves. Note other branches radiating from the ganglia, many of which go to form the aortic plexus; others, broken, pass to the more distal sympathetic ganglia, removed with the viscera; others cross the vertebral column and connect with the ganglia of the opposite side (commissural branches). Note that the spinal nerves of the thoracic or mid-region of the body pass singly to their distribution to the tissues, while those of the arm region and those of the caudal end of the axis unite with each other to form the brachial and lumbar plexuses respectively. Sketch one or two of the spinal nerves of the thoracic region in position, showing the corresponding portions of the sympathetic trunk and ganglia with their several connecting branches.

2. Appearances of the **spinal cord in situ.** In addition to the fetal pig in the hands of each student, one or two formalin preserved, demonstration specimens of adult human spinal cords are displayed for comparison and reference.

(a) Dissection, pig. Place the fetus, dorsal aspect upward, with limbs extended. Make an incision in the dorsal mid-line along the entire length of the body including the head and neck, and remove the skin, muscles and other soft parts adjacent to the vertebral column, working laterally from the midline. Then, with small

bone forceps or strong scissors, expose the spinal cord by clipping each arcus vertebræ (process) as close to the intervertebral foramen as possible, taking care to avoid crushing or tearing the structures lying within the vertebral canal. The spinal cord, enclosed in its dura mater, being thus exposed, carefully expose the spinal nerves of one side, dissecting sufficiently to show the spinal ganglia and the adjoining portions of the nerve trunks.

Also expose the encephalon by removing the superior cranial bones, taking care to leave intact the dura mater encephali.

(b) Sketch the dorsal aspect of the central nervous system *in situ* and enclosed by the meninges. Identify the following divisions: cerebrum, cerebellum and medulla oblongata with the fourth ventricle and its calamus scriptoris; the spinal cord, its general shape and extent, its cervical portion with cervical enlargement (intumescentia) and 8 pairs of cervical nerves attached; its thoracic portion with 12 pairs of thoracic nerves; its lumbar portion with the lumbar enlargement and 5 pairs of lumbar nerves, and its sacral portion with 5 pairs of sacral and one pair of coccygeal nerves. Compare with other specimens in the class for variations in the number of spinal nerves.

(c) **Meninges** of the spinal cord. Carefully slit the spinal dura mater down the dorsal mid-line. Does it vary in thickness? Compare with human. Lift aside the dura and identify the spinal arachnoidea (very scant in the fetus). In this a definite membrane? Subdural cavity and denticulte ligaments? What is the office of the subarachnoideal septum? Identify the spinal pia mater. How does it differ from the other two meninges in thickness and in its relation to the spinal cord? Make a sketch showing the relations of the three membranes to each other and to the spinal cord as seen in the human specimen.

Leaving the nervous system and its meninges *in situ*, place the entire specimen into several times its volume of 5 per cent. formalin for 12 hours or more.

(d) Wash in water and then expose the spinal cord proper along its entire length by pinning aside the dura mater. Note that in the fetus the cord occupies practically the entire vertebral canal. Compare with the adult human. Observe that each spinal nerve is connected with the spinal cord by a dorsal or posterior root and a ventral or anterior root. In which region are the roots shortest and attached most nearly at right angles to the cord? In which are they longest? What constitutes the cauda equina? Note that throughout, each root, upon approaching the cord, is divided into numerous radicular filaments. Examine the sacral extremity of the spinal cord, identifying the conus medullaris and the filum

terminale. How far does the latter extend? Is it nervous? Explain the fact that in the adult the spinal cord does not extend the entire length of the vertebral canal, and explain the increasing length of the spinal nerve-roots in passing candad, and the resulting cauda equina. From the human specimen, hardened in formalin, sketch the region of the cauda equina, showing the lumbar enlargement, conus medullare and filum terminale.

3. Sever the spinal cord of the pig from the encephalon at the level of the calamus scriptorius and carefully remove it from the vertebral canal, leaving attached one or two of the spinal nerves of each of the three regions. Then remove the spinal dura mater and arachnoidea and compare in detail the ventral aspect of the spinal cord with the dorsal aspect. Are the ventral roots thicker than the dorsal? Which are attached to the cord nearer the mid-line? Which set of the roots is more finely divided into radicular filaments? Note the anterior spinal artery. Are there posterior spinal arteries? Note the evident ventral or anterior median fissure and the line indicating the dorsal or posterior septum. Determine the boundaries of the posterior funiculus, the lateral funiculus and the anterior funiculus. Sever the head of the fetus from the body and return the head to the formalin solution for use in the study of the sense-organs.

4. The composition of the spinal nerves. Note that the trunk of each nerve is formed by the fusion of its dorsal and ventral roots in the neighborhood of its spinal ganglion. Detach a cervical or a thoracic nerve from the spinal cord, carefully clean it off under the dissecting microscope, and sketch it from its lateral aspect, showing the relative size of the two roots, their fusion and relation to the spinal ganglion, the relative size and direction of the anterior and posterior primary divisions of the nerve trunk and the connection of the ramus communicans. Tease a root and then the spinal ganglion. Differences between them? Which root is more intimately associated with the ganglion? Functions of the roots?

5. Sections of the spinal cord, unstained.

With a razor or sharp scalpel, remove a transverse section from the level of the seventh cervical segment. Keeping its orientation in mind, examine one of the cut surfaces for the following: the bilateral symmetry of the section; the posterior septum and the anterior median fissure; the shape of the column of gray substance and the variations in the thickness of the mantle of white substance surrounding it; the dorsal and ventral horns (columns), the gray commissure, and the relation of the respective nerve-roots to the horns. What constitutes the difference between white and gray substance? What is the reticular formation? What is the relation of the tissues of the pia mater to the section? Compare the section with similar sections through the thoracic and

lumbar regions. Were the mantle of white substance removed from the entire spinal cord, of what shape would the column of gray substance appear?

B. **Microscopic** Adult human. Stained sections.

1. Review the study of the neurones of the spinal cord made when considering the histology of the nervous system.

(a) Their external morphology, variations in size, significance and distinguishing features of the axons and dendrites, and the number of types of neurones represented in the spinal cord and spinal ganglia.

(b) Their internal morphology, the anatomy and relative size of the nucleus, the occurrence, origin and significance of the tigroid masses, and the variations, distribution and significance of the neurofibrillæ.

(c) The presence, extent and structure of the medullary sheaths.

(d) The forms of nerve termination, both central and peripheral, and the arrangements and relationships whereby the grouping and chaining together of neurones is accomplished. What is the significance of the "intermediate neurone?" Differences btween centrifugal and centripetal neurones?

2. Review carefully the architecture of the spinal ganglion. What characterizes the chief type of cell-body of the neurone contained in the ganglion? Character and functional significance of the Dogiel spinal ganglion "neurone of Type II?" Significance of the two other types of neurones represented? The occurrence and explanation of the excess of nerve-fibres of the peripheral side of the spinal ganglion as compared with the sum of the fibres contained in the dorsal and ventral roots? What is the behavior of the fibres of the dorsal roots upon their entering the spinal cord?

3. Under a magnification of 7 diameters, make a careful drawing of a transverse section taken from the sixth cervical segment (cervical enlargement) of the human spinal cord, stained by the Weigert method for the medullary sheaths and lightly counterstained with Upson's carmin for the cell-bodies of the neurones. Identify and show the following:

(a) The *meninges;* the anterior and posterior spinal arteries and veins; the posterior median sulcus, posterior septum, and anterior median fissure; the postero-lateral, antero-lateral, postero-intermediate and antero-intermediate sulci and the septa indicated by them. Which of the sulci are associated with the entrance and exit of the fibers of the dorsal and ventral roots? Note the septum subarachnoideale. How is the subarachnoid space divided? Relation of the anterior spinal artery to the ventral fissure and the "linea splendens?"

(b) The *gray substance;* the dorsal horn (posterior column) with its apex, caput and cervix; the reticular formation; the ventral horn (anterior column), lateral horn, and gray commissure with the central canal, central gray substance, and ventral (anterior) white commissure. State the origin and distribution of the axones which constitute the ventral white commissure? What is the relation of the dorsal horn to the fibres of the dorsal roots? Of the ventral and lateral horns to the fibres of the ventral roots? What is the structure and appearance of the gelatinous substance of Rolando? Note the distribution of the larger cell-bodies in the ventral gray substance and divide them into—

(1) A ventral group with a ventro-lateral and a ventro-medial portion.
(2) A dorso-medial group.
(3) A lateral group occupying the lateral horn, and sometimes separated into a dorso-lateral and a ventro-lateral portion; and
(4) An intermediate group, occupying the mid-dorsal portion of the ventral horn. State the probable distribution of the axones arising from each of these groups of cell-bodies.

How do the cell-bodies of the dorsal horn differ from those of the ventral gray substance? What is the general functional significance of the smaller cell-bodies scattered throughout the entire gray substance?

(c) The *white substance.* Note that the axons of the white substance comprise four general neurone systems: (1), the cerebro-spinal system ascending and descending; (2) the cerebello-spinal system, ascending and descending; (3), the commissural fibers which cross the mid-line to connect the two sides, and (4), the association fibers which course varying distances both upward and downward to connect different levels of the gray substance and which compose the fasciculi proprii of the spinal cord. Give the boundaries of the three funiculi into which the white substance is divided and ascertain the position, approximate shape, relative size, origin and functional direction of the fasciculi composing each funiculus, as follows:

(1) The posterior funiculus, consisting of the fasciculus gracilis (Goll's column), the fasciculus cuneatus (Burdach's column), the comma-shaped fasciculus, the dorsal fasciculus proprius, and the cornu-commissural tract.
(2) The lateral funiculus, consisting of the lateral cerebro-spinal fasciculus (crossed pyramidal tract), the ascending cerebello-spinal fasciculus (direct cerebellar tract), the superficial ventro-lateral fasciculus (Gower's tract), the olivary fasciculus (Helwig's bundle), the mixed intermediate fasciculus, the marginal fasciculus of Lissauer, and the lateral fasciculus proprius.

(3) The anterior funiculus, consisting of the ventral cerebro-spinal fasciculus (direct pyramidal tract), the anterior marginal fasciculus, the sulco-marginal fasciculus, the ventral fasciculus proprius, and the commissural bundle.

Attach the names to all structures represented in the drawing.

4. Under the same magnification, make careful drawings of similarly treated sections taken:

(a) From the first cervical segment.

(b) From the eighth thoracic segment.

(c) From the middle of the lumbar enlargement.

(d) From the conus medullaris.

What is the position and longitudinal extent of the nucleus dorsalis (Clark's column)? To what fibres do its cell-bodies give origin? In which segments is it thickest? What is the position, significance and extent of "Stilling's nucleus?" Attach the names to all structures not found in the first section or which are greatly modified.

5. Lay the five drawings in serial order and by comparisons verify the following statements:

(a) In the conus medullaris (sacro-coccygeal region), the gray substance is surrounded by a comparatively thin mantle of white substance. The dorsal horns are nearly as thick as the ventral horns and the gray commissure is relatively thicker than in any of the other regions. Is there a lateral horn? Why should the white substance be relatively less abundant here, and what axone systems predominate in it?

(b) In the lumbar region, the white substance begins to predominate and the number of large cell-bodies here is relatively large. Why? Is there a lateral horn? Compare the posterior funiculi here with those of the other sections and explain their differences. Note the shape of the section.

(c) The thoracic region is characterized by its relatively small amount of gray substance. The ventral and dorsal horns together form two slender crescents united across the mid-line by a thin gray commissure situated relatively nearer the ventral side of the section. The nucleus dorsalis modifies the shape of the cervix of the dorsal horn. Compare the shape and size of its cells with those of the ventral horn. What fasciculus arises from it? Which of the cell groups mentioned above are represented in the ventral horn and what muscles are supplied by their axones? Especially to be noted is the increase of the posterior funiculi. Is the white substance of the entire section absolutely, as well as relatively, greater than in the sections from the more caudal regions? Why? Is the reticular formation evident here?

(d) The cervical region shows a decided increase in the absolute amount of white substance. Why? The fasciculus gracilis can be easily distinguished from the fasciculus cuneatus and the lateral fasciculi, in the region of the enlargement, have so increased as to give the section an oval shape. The ventral horns are much thicker in the enlargement than in the thoracic region and there is a well marked lateral horn. Why? The section from the first cervical segment shows the gray substance reduced to a figure similar to that in the thoracic region. The amount of white substance here is also somewhat decreased. Why should it decrease at all? Explain the production and significance of this enlargement of the spinal cord. Why do both the ascending and descending fasciculi show increase in passing from the conus medullaris towards the encephalon? Do all the fibres of the dorsal roots of the spinal nerves reach the brain? Explain the variations in the amount of the fasciculi proprii at the different levels.

6. Under higher magnification, draw the gray commissure of the section from the lumbar region, showing in detail the central canal, the central gelatinous substance and the ventral white commissure. What is the origin and distribution of the fibres composing the latter? Is there a dorsal white commissure? Are the ependyma cells ciliated? Is the lumen of the central canal maintained throughout the spinal cord?

II. THE ENCEPHALON (brain).

Human brains removed and preserved in formalin by the embalmer, and fresh sheep heads, obtained from the butcher, will be supplied.

A. In situ. Fresh. Sheep's Brain. Introductory Exercise.

1. Removal.

(a) Grasp the sheep head firmly in a vise, cranium upward, and with a saw make a transverse cut involving the anterior rims of the two orbits. Then, along each side of the head, beginning each cut by sawing obliquely through the occipital condyle into the lateral portion of the foramen magnum and thence, let the line of the saw, slanting slightly inward, pass close to the orbit and join the transverse cut. Work lightly and do not let the saw cut into the cranial cavity. With chisel or skull-wrench break the vault of the cranium loose and carefully lift it from behind and remove it, freeing the adherent dura mater from the bone with a scalpel handle and taking care not to injure the cranial contents. With blunt-pointed scissors, cut the dura mater along each side just below the line of the saw.

(b) With scalpel handle, free each olfactory bulb from the lamina cribosa of the ethmoid bone, noting the filaments of the olfactory

nerve passing through the foramina of the lamina and entering the ventral aspect of the bulb. Then, beginning from behind, leaving the dura mater adherent to the floor of the cranium, lift the medulla oblongata and, as the cerebral nerves come into view, sever each pair as close to their respective foramina as possible. The hypophysis is situated in a pocket of the dura mater (diaphragma sellæ) in the hypophyseal fossa of the cranium and may be cut out with the scalpel without breaking the infundibulum by which it is attached to the brain. The optic nerves must be severed near the optic chiasma, at their exit from their foramina. Lay the specimen in a dissecting pan, dorsal surface upward.

2. The Meninges.

(a) Gently lift a portion of the dura mater *in situ* on the dorsal surface of the encephalon and identify the sub-dural cavity, the arachnoidea of the encephalon, the subarachnoid cavity, the pia mater of the encephalon, and the subarachnoid trabeculæ connecting the arachnoidea and pia. What is the significance of the latter? Note that the dura mater consists of two closely related layers, the outer of which serves as the internal periosteum of the cranial bones. The two layers are separable in occasional localities and between them lie veins, venous lacunæ and venus sinuses. Explain the absence of two layers in the spinal dura mater, and compare the epidural cavity of the vertebral canal and its venous plexus with the spaces between the two layers here and the venous channels contained. Test and explain the fact that the dura mater is more firmly adherent to the bone in the floor of the cranium than to the vault.

(b) Remove the dura mater from the dorsal surface of the encephalon, and, comparing with a demonstration specimen of the human, note the following infoldings or duplications of its inner layer:

(1) The falx cerebri, extending into the longitudinal fissure between the cerebral hemispheres and containing the superior and inferior sagittal sinuses, and, posteriorly, the sinus rectus, running along the line of its junction with,

(2) The tentorium cerebelli, extending into the transverse fissure between the cerebrum and the cerebellum, its superior border containing the tentorial notch (incissura) which saddles the mesencephalon.

(3) The falx cerebelli, present in the human specimen, extending in the groove (cerebellar notch) between the cerebellar hemispheres, but absent in the sheep for the reason that the cerebellar hemisphere are less developed.

(4) In the floor of the cranium, determine the relations of the diaphragma sellæ to the hypophyseal fossa, the hypophysis and the

infundibulum, and dissect out one of the semilunar (Gasserian) ganglia, determining the formation and position of the "Meckel's caves."

(c) Of the arachnoidea of the encephalon, determine the boundaries and contents of the cerebello-medullary cistern, the cistern of the lateral fossa of the cerebrum, and the chiasmatic and interpeduncular cisterns. What are the arachnoid granulations (Pacchionian bodies) and their relations to the cerebrospinal lymph?

(d) Note that the pia mater of the encephalon is closely applied to its surface throughout and sends vascular duplications into its two great fissures:

(1) The tela chorioidea of the fourth ventricle, lying in the transverse cerebellar fissure and roofing over the inferior portion of the fourth ventricle, and

(2) The tela chorioidea of the third ventricle (velum interpositum), extending into the transverse cerebral fissure and roofing over the cavity of the third ventricle.

Now remove the mandible with the muscles attached and place the remainder of the sheep's head into 5 per cent. formalin (2½ per cent. formaldehyde) that it may be preserved for use in the study of the organs of special sense.

3. General topography of the encephalon (sheep).

(a) Identify and note the position of the following:

(1) The frontal, temporal and occipital poles of the cerebrum; the lateral cerebral fissure, the temporal lobe, and hippocampal gyrus; the hypophysis, the infundibulum and tuber cinereum; the mammillary bodies, peduncles of cerebrum (crura cerebri) and interpeduncular fossa; the pons with basilar sulcus and brachia of pons; the cerebellar hemispheres with floccular lobes and the superior and inferior vermis; the medulla oblongata with anterior (ventral) median fissure, pyramids, decussation of the pyramids, olives, lateral and posterior (dorsal) funiculi, and restiform bodies. What is the relation of the pons and restiform bodies to the cerebellum? Note fibres crossing each restiform body (external arcuate fibres) giving it the appearance which suggests its name and the very evident trapezoid body of the sheep.

(2) Press open the longitudinal fissure of the cerebrum and note the corpus callosum. Of what substance is it composed and what is its evident significance?

(3) Sever the corpus callosum along the mid-line and note the longitudinally disposed and paired bodies of white substance, the fornix. Below the fornix, observe the paired masses of gray substance, the thalami, covered by the non-nervous tela chorioidea

of the third ventricle. Connecting the corpus callosum with the fornix, note the vertically placed septum pellucidum.

(4) Press apart the occipital poles and the cerebellum and note the quadrigeminate bodies (the mesencephalon) with the epiphysis (pineal body) resting upon the superior colliculi. Distinguish the anterior medullary velum extending from the inferior colliculi and forming the roof over the anterior portion of the fourth ventricle.

(b) On the *basal surface*, identify the 12 pairs of cranial nerves, giving the number and name of each, and its distinguishing peculiarities. What can be said especially of the olfactory nerve? With what is the tuber cinereum continuous? Note the inferior extension of the longitudinal fissure of the cerebrum.

(c) **Ventricles of the encephalon.**

(1) Divide the cerebellum along the vermis, taking care not to injure the structures below it, and remove one of the halves, observing and naming the structures severed in the removal. Note the chorioid tela and plexus of the *fourth ventricle* and the rhomboid fossa constituting the floor of the ventricle, with its pointed, inferior termination, the calamus scriptoris, at which the ventricle is continuous into the central canal of the medulla oblongata and spinal cord. Observe that the superior, or anterior, portion of the fourth ventricle extends under the anterior medullary velum and, with a probe, determine that this is continuous with the third ventricle by way of the aqueduct of the cerebrum (aqueduct of Sylvius) which passes under the quadrigeminate bodies.

(2) The *third ventricle*, covered by its chorioid tela and continuous into the lateral ventricles of the cerebrum by way of the two interventricular foramina (foramina of Monro). Note the gray, intermediate mass (middle commissure) connecting the thalami across the ventricle.

(3) Tear one of the cerebral hemispheres, noting the shape of the lateral ventricle with its chorioid plexus, its anterior horn, central part, posterior horn, and inferior horn. What is the chorioid plexus and into which of the horns does it extend? Note the caudate nucleus. Its structural nature, its extent, and why its name?

(d) Identify the following general divisions of the encephalon.

(1) Rhombencephalon.

(i) Medulla oblongata.

(ii) Metencephalon { cerebellum. / pons.

(iii) Isthmus of rhombencephalon.

(2) Cerebrum.

(i) Mesencephalon { quadrigeminate bodies.
cerebral peduncles.

(ii) Prosencephalon { diencephalon (thalami, etc.).
telencephalon (cerebral hemispheres, etc.).

B. The Human Encephalon (*detailed study*).

About twelve hours after the process of embalming and injection of the blood-vessels of the subjects for the dissecting room, the brains are removed and preserved separately in 10 per cent. formalin. They should be washed in water for at least one hour before beginning work on them, should be kept moist while working and, between each period of work, should be kept in a 2 per cent. solution of formalin.

1. *External features.*

(a) Identify all the structures mentioned in A, 3, (a), (1) and (2) above. Explain the difference in the direction of the human medulla oblongata from that of the sheep. As compared with the sheep's brain, note the more distinct pyramids and olives, the relatively larger cerebellum and pons, the more distinctly separated mammillary bodies, the relatively much larger cerebrum with much more developed frontal and temporal regions and more marked lateral cerebral fissures; the much less developed olfactory bulbs and tracts but the more distinct olfactory trigone. Carefully examine the remains of the arachnoidea and note variations of the pia mater upon the dorsal and ventral aspects of the brain. How does the pia in the sulci differ from that on the summits of the gyri?

(b) **Superficial blood-vessels.**

Note that the arteries of the brain approach and the veins leave it from its ventral aspect. Its arterial supply is derived from the two vertebral and the two internal carotid arteries. Of the larger vessels trace the following:

The two vertebral arteries unite near the inferior border of the pons to form the single basilar artery. This passes along the pons in its basilar sulcus and gives off the inferior and superior cerebellar arteries and the smaller rami to the pons. At the superior border of the pons, the basilar artery bifurcates into the two posterior cerebral ateries, which branch repeatedly to spread over the occipital regions of the cerebral hemispheres. Near their origin, the posterior cerebral arteries give off the posterior communicating arteries, one for each side, which pass forward, encircling the mammillary bodies and the tuber cinereum, and then join the internal carotid arteries at the outer sides of the optic chiasma.

Each internal carotid artery divides into three branches:

(1) The chorioid artery, the smallest, passes dorsally, around the peduncles of the cerebrum and inward to the chorioid plexuses of the ventricles.

(2) The middle cerebral artery, the largest, breaks up into its larger branches in the lateral cerebral fissure and spreads over the lateral surface of the brain.

(3) The anterior cerebral artery passes forward into the ventral part of the great longitudinal fissure and becomes the chief supply of the frontal and medial portions of the cerebral hemisphere.

The two anterior cerebral arteries are connected across the mid-line just in front of the chiasma, by the single, short anterior communicating artery. Thus a circle ("the circle of Willis") is formed, enclosing the chiasma, the tuber cinereum and the mammillary bodies. Enumerate the branches which contribute to the circle. Work out the names and distribution of the principal branches of the arteries named.

The *external veins* of the encephalon lie, for the most part, superficial to the arteries and course in the arachnoidea and dura mater and, therefore, their study must be made chiefly from the foramina and remaining meninges within the cranium of the subject in the dissecting room. The veins arise within the substance of the brain and the chorioid plexuses, converge into larger branches in the pia mater and subarachnoid space, pass through the arachnoid and empty into the venous sinuses and lacunæ situated between the two layers of the dura mater. The large transverse sinus is situated in the tentorium cerebelli. It is joined in the mid-line by the superior sagittal sinus which runs the entire superior length of the falx cerebri and falx cerebelli and is fed by the various superior cerebral and superior cerebellar veins. The inferior sagittal sinus runs in the inferior border of the falx cerebri and, in the tentorium cerebelli, is continuous into the straight sinus, which opens into the transverse sinus near the juncture of this with the superior sagittal sinus. In each of the lateral ventricles, the chorioid vein, assembled along the chorioid plexus, and the terminal vein, assembled along the border of the caudate nucleus, unite with each other at the interventricular foramen to form one of the internal cerebral veins. The two internal cerebral veins (veins of Galen) pass backward in the tela choroidea of the third ventricle, receiving the basal veins and smaller twigs, and unite in the mid-line to form the vena magna of the cerebrum, which latter joins the straight sinus at its junction with the inferior sagittal sinus.

The lateral limbs of the transverse sinus (lateral sinuses) empty

into the internal jugular veins and thus the blood passes out of the cranium at either side. In the dura mater of the floor of the cranium, the two cavernous sinuses empty into the internal jugular veins by way of the two inferior petrosal sinuses.

(c) **The Cranial Nerves.**—Give special attention to the relative size, form, locality of attachment, origin and function of the fibers of each of the twelve pairs:

(1) The *Olfactory* arises in the olfactory region of the nasal epithelium and terminates in the olfactory bulb. Sensory.

(2) The *Optic* passes obliquely medianward from the ocular bulb to the optic chiasma; thence, as *optic tract*, its fibers pass around the cerebral peduncle to the thalamus (pulvinar), the lateral geniculate body and the superior colliculi. Sensory. Why may it be considered an intercerebral tract instead of a typical nerve?

(3) The *Oculomotor* emerges in its sulcus in the medial border of the cerebral peduncle and through the posterior perforated substance of the interpeduncular fossa. Motor.

(4) The *Trochlear*, smallest of the cranial nerves, arises from the dorsal aspect of the isthmus of the rhombencephalon near the inferior border of the inferior colliculi (frenulum of anterior medullary velum), passes downward around the cerebral peduncle and comes into view on the ventral aspect of the brain near the superior margin of the pons. Motor.

(5) The *Trigeminus*, largest of the cranial nerves, consists of two roots: The sensory root or major portion of the nerve enters the rhombencephalon, piercing the superior and lateral aspect of the pons. The small, cylindrical, motor root or minor portion emerges through the lateral aspect of the pons close to the superodorsal margin of the sensory root. In emerging, its fibers mingle with those of the sensory root, but soon assemble into the distinct bundle which then loops around the medial margin of this root. Shape and location of the ganglion of origin of the major portion? Name and direction of the three nerves which arise from it? Which of them is joined by the motor root?

(6) The *Abducens* leaves the medulla oblongata at the inferior margin of the pons in the transverse fossa between this margin and the base of the pyramid and near the ventral median fissure (foramen cecum), and runs forward and lateralward over the surface of the pons. Motor.

(7) The *Facial*, larger nerve than the abducens, appears in the transverse fossa at the inferior margin of the pons, lateral to the emergence of the abducens. It consists of two roots, a large motor root and a small sensory root, or pars intermedia (interme-

diate nerve), which latter comprises its more lateral portion. The fibers of the two roots usually blend so that the double character of the nerve is more or less concealed in its intracranial portion and, when separated beyond, the smaller root carries some motor fibers. Mixed. What and where situated is the ganglion of origin of the sensory fibers?

(8) The *Acoustic* (*Auditory*).—Large nerve which enters the rhombencephalon lateral from but near the facial and at the inferior margin of the brachium of the pons, superior and dorsal to the olive. It consists of two roots or divisions: The vestibular root (*vestibular nerve*), the more medial of the two roots, enters on the ventral side of the restiform body and dorsal to the line of the olive. The more lateral, cochlear root (*cochlear nerve*), in enering, arches around the dorsal and outer surface of the restiform body and some of its fibers course in the floor of the fourth ventricle. Sensory. Where are the ganglia of origin of the separate roots? Why could the two roots be considered as separate cranial nerves?

(9) The *Glossopharyngeal*.—Attached to the medulla oblongata in five to seven root-filaments in the superior part of the sulcus between the olive and restiform body and in line, longitudinally, with the facial nerve. Mixed nerve, though when traced outward, all the filaments blend into a single trunk in which are situated two ganglia of origin of its sensory fibers. Names and locations of the ganglia?

(10) *The Vagus* (*Pneumogastric*).—Attached to medulla in ten to fifteen root filaments, in the same sulcus, inferior to and in line with those of the Glossopharyngeal. Filaments, when followed, converge into a single stout, cylindrical trunk. Like the ninth, it is a mixed nerve whose sensory axons arise in two ganglia interposed in its trunk. Names and situation of the ganglia?

(11) The (*Spinal*) *Accessory*.—Emerges from the lateral aspect of the medulla oblongata and the upper four to six segments of the spinal cord in a series of rootlets which contribute to form a trunk which runs upward and parallel to the lateral surface of the medulla. Consists of an *accessory* or *superior part*, consisting of the three to six rootlets arising from the medulla in line with and below the filaments of the vagus, and a *spinal* or *inferior part*, the remaining rootlets which emerge from the lateral funiculus of the spinal cord, dorsal to the denticulate ligament and between the lines of attachment of the dorsal and ventral roots of the spinal nerves. Motor. Distribution and relation to the vagus?

(12) The *Hypoglossus*.—Emerges from the medulla oblongata in ten to sixteen root filaments, between the pyramid and the olive,

in the upper end of the ventro-lateral sulcus, and in line with the ventral root filaments of the spinal nerves. Root filaments usually first converge to form two bundles which later unite into the single trunk of the nerve. Motor.

Note that, exclusive of the olfactory and optic, the cranial nerves are attached to the encephalon along two different lines: a ventro-lateral line and a lateral line. Make a tabulation of the twelve pairs, comparing their functions, the character of their attachment, their size and the lines of their attachment.

(d) General study of the **base of the encephalon.**

Remove the blood-vessels of the base of the encephalon and carefully peel off the pia mater from the structures near the mid-line, taking care not to injure the nerve-roots and the tuber cinereum. Whence do the nerves obtain their sheaths? Look up the peculiar nature of the sheath of the optic nerve. Observe that the olfactory tract runs backward from the olfactory bulb in the olfactory sulcus, between the orbital gyri and the gyrus rectus, and terminates in the olfactory trigone, the three striæ of which disappear in the anterior perforated substance. What is the apparent nature of the anterior and posterior perforated substances and what are the perforations? What is the apparent course of the fibers of the cerebral peduncles and what is their function? Study the course of the fibers of the pons as they pass into the brachium pontis of either side. What factors produce the basilar sulcus? Course of the oblique fibers of the pons? How do the gyri of the cerebral hemispheres differ from those of the cerebellum? Shape and gyri of the flocculus? Make a line drawing of the entire base of the encephalon, exclusive of the blood-supply, showing and naming the important features.

2. The divisions of the encephalon.

(a) The Cerebrum.

(1) *The Prosencephalon.*

(i) *Medial Surface.*—With sharp scalpel cut one of the cerebral peduncles transversely just posterior to the mammillary body, taking care not to injure the mesencephalon. Then turn the specimen convex surface upward, press open the longitudinal fissure and, with brain knife, carefully divide the corpus callosum along the mid-line. Continue the incision so that the knife divides the fornix, severs the intermediate mass, passes between the mammillary bodies, splits the tuber cinereum, and passes through the middle of the optic chiasma. Carefully remove sufficient of the pia mater from the hemisphere thus detached, then lean it slightly lateralward on its ventral

surface and make a drawing of the entire medial surface and medial portion of the ventral surface of prosencephalon, showing the shape and position of all the structures in view and attach their names. Indicate the four parts of the corpus callosum and give careful attention to the names, position and extent of the various sulci to be seen. Are the sulci of the two hemispheres alike? What are the relations of the septum pellucidum, lamina rostralis and lamina terminalis to the corpus callosum. Relation of the fornix to the mammillary body? Relation of the columns of the fornix to the interventricular foramina? What is the tenia of the fornix? Structure of the chorioid tela of the third ventricle? Shape of the third ventricle and realtion of the infundibulum and tuber cinereum to its cavity? Compare the function of the anterior commissure with that of the corpus callosum. Slit the septum pellucidum and note the caudate nucleus in the lateral ventricle. Ascertain the shape and disposition of this nucleus from text illustrations. Note the shape of the thalamus. What is the pulvinar thalami?

(ii) Identify the two **divisions of the prosencephalon,** determining the position and relationship of the parts composing them as follows:

The *diencephalon*, comprising the thalamencephalon (thalamus, metathalamus and epithalamus) and the mammillary portion of the hypothalamus, and,

The *telencephalon*, comprising the optic portion of the hypothalamus, the optic nerves (and retina), and the cerebral hemispheres (striate bodies, olfactory bulbs and tracts, and the cerebral cortex).

To which of these divisions do the epiphysis (pineal body) and the hypophysis (pituitary body) belong? Identify and ascertain the significance of the habenulæ, commissure of habenulæ, pineal recess, medullary striæ and tenia of thalamus. What function of the pulvinar and the geniculate bodies is apparent?

(iii) The **Lobes of the Cerebrum** (telencephalon).—First, for the purposes of orientation, fix in mind the name and extent of the different borders of the hemispheres, and as land marks, carefully identify the central sulcus (fissure of Rolando), the parieto-occipital fissure, the lateral (Sylvian) fissure, the hippocampal fissure, and the parts of the sulcus singuli, and then determine the extent and component parts of the lobes as follows:

(1a) *The Frontal Lobe.*—Convex surface: the anterior central gyrus, the superior and middle frontal gyri, the inferior

frontal gyri with opercular, triangular and orbital parts, and the sulci separating these gyri. Medial surface: superior frontal gyrus, paracentral lobule and rostral sulcus. Ventral surface: gyrus rectus, olfactory sulcus, and gyri and sulci orbitales.

(2a) *The Parietal Lobe.*—Convex surface: posterior central gyrus, superior and inferior parietal lobules, the latter containing the supramarginal and angular gyri, and the sulci separating these parts. Medial surface: the precuneus and a portion of the paracentral lobule.

(3a) *The Occipital Lobe.*—Convex surface: superior and lateral occipital gyri and sulci. Medial surface: the cuneus, the calcarine fissure and the posterior extremity of the lingual gyrus. Tentorial surface: posterior portions of the lingual and fusiform gyri with that part of the collateral fissure which lies between them.

(4a) *The Temporal Lobe.*—Separated from the frontal and parietal lobes by the lateral fissure of the cerebrum and comprising the superior, middle and inferior temporal gyri and sulci, and the anterior portions of the fusiform and lingual gyri with the collateral fissure between them.

(5a) *The Island* (central lobe).—Expose by pressing apart the lips of the lateral fissure (operculum) and note the circular sulcus, short gyri, long gyrus and threshold of the island. Explain the processes of development which have resulted in the position and configuration of this lobe.

(6a) *The Rhinencephalon* (olfactory lobe and limbic lobe).—Olfactory bulb, tract and trigone (name the striæ); parolfactory area, subcallosal gyrus, anterior perforated substance, gyrus fornicatus; the fornix, the mammillary body and a part of the septum pellucidum. Name and identify the parts of the complicated gyrus fornicatus. Give the reasons for the names given the different parts of the hippocampus. Look up the origin and course of the thalamomammillary fasciculus and the destination of each of the olfactory striæ.

Explain the formation of the gyri and sulci of the encephalon in general. Which are the first to appear? Name the parts of the operculum and the gyri which contribute to each part. What is the somesthetic area and the gyri forming it? Give the component parts of the cortical areas with which each of the organs of special sense is chiefly concerned. Extent and components of the so-called association centers?

(2) *The Mesencephalon.*

From which of the primary cerebral vesicles is this second division of the cerebrum derived? Determine the position and appearance of the following structures comprising it. Dorsal surface: the superior and inferior colliculi of the corpora quadrigemina, the brachia of each and the furrows separating them. Ventral surface: peduncles of cerebrum, anterior and posterior recesses, and the posterior perforated substance. The aqueduct of the cerebrum passes through the mesencephalon.

(b) **The Rhombencephalon.**

(1) The *Cerebellum* and *Pons* (Metencephalon).

(i) Dorsal and lateral surface of the cerebellum: Note that the cerebellar hemispheres join each other in a medial ridge, the superior vermis, which disappears from view in the marked posterior cerebellar notch. Of the hemispheres, identify and name the four lobes apparent on the dorsal and lateral surfaces and the fissures separating them. Of the superior vermis, identify the central lobule, the monticulus, and the folium. How do the gyri and the sulci and the distribution of the superficial blood-vessels of the cerebellum differ from those of the telencephalon?

(ii) Ventral surface of cerebellum: Carefully sever the brachium of the pons (middle cerebellar peduncle) of the same side as that from which the cerebral hemisphere has been removed, then pass the brain knife vertically through the mid-line along the summit of the monticulus, taking care not to injure the floor of the fourth ventricle, and remove the half of the cerebellum by severing the anterior medullary velum close under the central lobule of the vermis. On the ventral surface of the half removed, identify, in addition to the lobes already named, the biventral and gracile lobules, the tonsila, the flocculus, and the inferior vermis (divided), the latter being separated from the hemispheres on either side by the vallecula and divided by transverse fissures into the tuber, pyramid, uvula, and nodula of the vermis. Are there secondary flocculi? Identify the peduncle of the flocculus and the posterior medullary velum. Where and what is the ligula?

In the cut surfaces identify arbor vitæ, medullary body medullary lamina, cortical substance, and, in addition to the middle peduncle, determine the position of the brachium conjunctivum (superior peduncle) and the restiform body (inferior peduncle). Function of each of the three peduncles? What is the orientation of the cells of Purkinje with reference to the

cortical substance and to the course and contour of the gyri of the cerebellum?

Make a careful *drawing of* the convex lateral surface of the *entire encephalon* from the side remaining intact and attach the names to all the parts in view.

(2) The *Medulla Oblongata.*

(i) Dorsal and lateral surfaces: Note that the posterior sulcus of the spinal cord becomes deepened into the posterior median fissure of the medulla oblongata, which latter is continuous into the calamus scriptorius, and that the line of attachment of the chorioid tela of the fourth ventricle is indicated by a torn ridge of slightly thickened pia mater, the tænia of the fourth ventricle, which crosses the mid-line at the junction of the posterior fissure with the calamus scriptorius, producing a more or less distinct bridge over the tip of the calamus scriptorius, known as the obex. Identify the postero-intermediate and the postero-lateral sulci; the funiculus gracilis and funiculus cuneatus (why funiculus here?) terminating in slightly bulbous eminences, viz., the nucleus of the funiculus gracilis (clava) and the tuberculum cuneatum. Trace the lateral funiculus of the spinal cord into the restiform body and note that the latter in passing toward the brachium of the pons so increases in size as to become the chief cause of the decided upward increase in the width of the medulla oblongata. Identify the course of the fibres of the acoustic nerve passing around the restiform body to form the acoustic medullary striæ in the floor of the fourth ventricle.

(ii) Again identify the more prominent structures composing the ventral surface of the medulla oblongata, already considered in the study of the ventral surface of the encephalon as a whole. On both the dorsal and ventral surfaces determine the position of the boundary lines separating the medulla oblongata from the metencephalon above and the spinal cord below.

(3) The *floor of the fourth ventricle* (rhomboid fossa).

Remove the half of the anterior medullary velum and with it a portion of the brachium conjunctivum of the side from which the hemispheres have been removed, and press the attached half of the cerebellum slightly outward so as to expose the entire floor of the fourth venricle. What is its shape and where and with what canal is it continuous? Determine the position extent and functional significance of the following:

The medial eminence, bounded by the median sulcus and the sulcus limitans, and consisting of the eminence of the nucleus

of the vagus (ala cinerea), the eminence (trigone) of the hypoglossus, the eminence of the facial and abducens (colliculus facialis), the region of the nucleus incertus, and the locus ceruleus; the acoustic tubercle and area with the acoustic medullary striæ; the area postrema and the funiculus separans. Where are the superior and inferior foveæ and what do they separate? Where is the region of the motor nucleus of the trigeminus? Of the lateral recess of the fourth ventricle? Note that the inferior, intermediate and superior portions of the floor of the ventricle belong each to a different division of the rhombencephalon. What are these divisions?

Make a careful drawing of the floor of the fourth ventricle, including the dorsal aspect of the medulla oblongata and the mesencephalon, and the cut surfaces of the rhombencephalon, naming all the prominent structures.

3. Sections of the encephalon.

General Directions.—In making the sections called for below, first carefully ascertain the place of each by determining two or more superficially indicated structures through which the knife is to pass and then, to avoid unevennesses of surface, make each section with one stroke of the knife. Use figures 23 and 24 as guides in determining the levels at which the sections are to be made. The larger sections require a knife with a thin and exceptionally long blade. The sections of the regions of the medulla oblongata, pons, mesencephalon and thalamencephalon will be supplemented by sections taken from corresponding levels of another specimen and stained by the Weigert method. In these cases study with a dissecting lens one of the cut surfaces exposed by the passage of the knife as directed, noting the position, appearances and interrelations of the structures mentioned under the respective sections, compare with the stained section and then, using the stained section under the dissecting microscope, make a careful drawing. Use the compound microscope for the more detailed structure of the stained section. In the larger sections through the cerebellar and cerebral hemispheres, the more important structures may be distinguished without the aid of stained preparations. With the cerebral hemisphere especially, the effect of a symmetrical section through the entire cerebrum may be obtained by turning the detached part so that the two cut surfaces exposed by a given passage of the knife are in plane with each other, the medial aspects facing together. Work with the specimen wet with water and, at the end of the period of study, return all the parts to the jar of dilute formalin. On each drawing, attach the names of all the structures identified, using dotted leaders radiating to the periphery of the drawings, where the names should be written parallel with each other. Number the drawings in series. Let the lowest section of the medulla oblongata be "Number 6" in order to include in the series the five sections of the spinal

cord already studied. Beginning with section nine, it will suffice to draw one half of each section with only a small strip of the opposite side of the mid-line included.

(a) *Sections of the rhombencephalon.*

Section 6.—To pass transversely through the decussation of the pyramids. Draw, showing the position, form and relation of the following:

Posterior median fissure (posterior septum of the spinal cord) and anterior median fissure; pyramids and decussation of pyramids; anterior columns, reticular formations, gelatinous substance of Ralando, central canal and central gray substance; funiculus gracilis with portion of its nucleus, funiculus cuneatus, internal arcuate fibers and the lemniscus (fillet); spinal tract of the trigeminus, lateral funiculus and root filaments of the spinal accessory nerve.

Carefully compare this drawing with those of the cervical region of the spinal cord noting all modifications resulting in the transition of the spinal cord into medulla oblongata. How has the position of the central canal changed? Explain the changes in the anterior and posterior columns (ventral and dorsal horns). Whence arise the axones taking part in the decussation of the pyramids and what is their relation to the lateral funiculi? Where do they terminate and to what neurones are they distributed? What is the lemniscus and the significance of the nuclei of the funiculus gracilis and the funiculus cuneatus? What areas of the section are occupied by the cerebello-spinal axones?

Section 7.—To pass through the inferior extremities of the olives and the point of the calamus scriptorius. Draw, giving special attention to the following:

Anterior median fissure, pyramids, raphe, calamus scriptorius and obex; nuclei of funiculus gracilis and of funiculus cuneatus, internal arcuate fibres and decussation of the lemnisci; commissural nucleus of ala cinerea, spinal tract of the trigeminus and the gelatinous substance containing its nucleus of termination; restiform body, external arcuate fibres, lateral nuclei and nucleus of the inferior olive.

What has become of the posterior median fissure and where is the central canal? How do the pyramids here differ from section 6, and why? Whence arise the internal and external arcuate fibres and what is their relation to the lemniscus? Relations of the spinal tract of the trigeminus and the distribution of axones arising from its nucleus? Significance of the decussation of the lemnisci and of the pyramids? Look for the nucleus of the hypoglossus.

Section 8.—To pass transversely through the middle of the olives. Let the section involve the overhanging portion of the cerebellar hemisphere but avoid cutting the cerebral hemisphere. Draw, exclusive of the cerebellum, showing the character of the following:

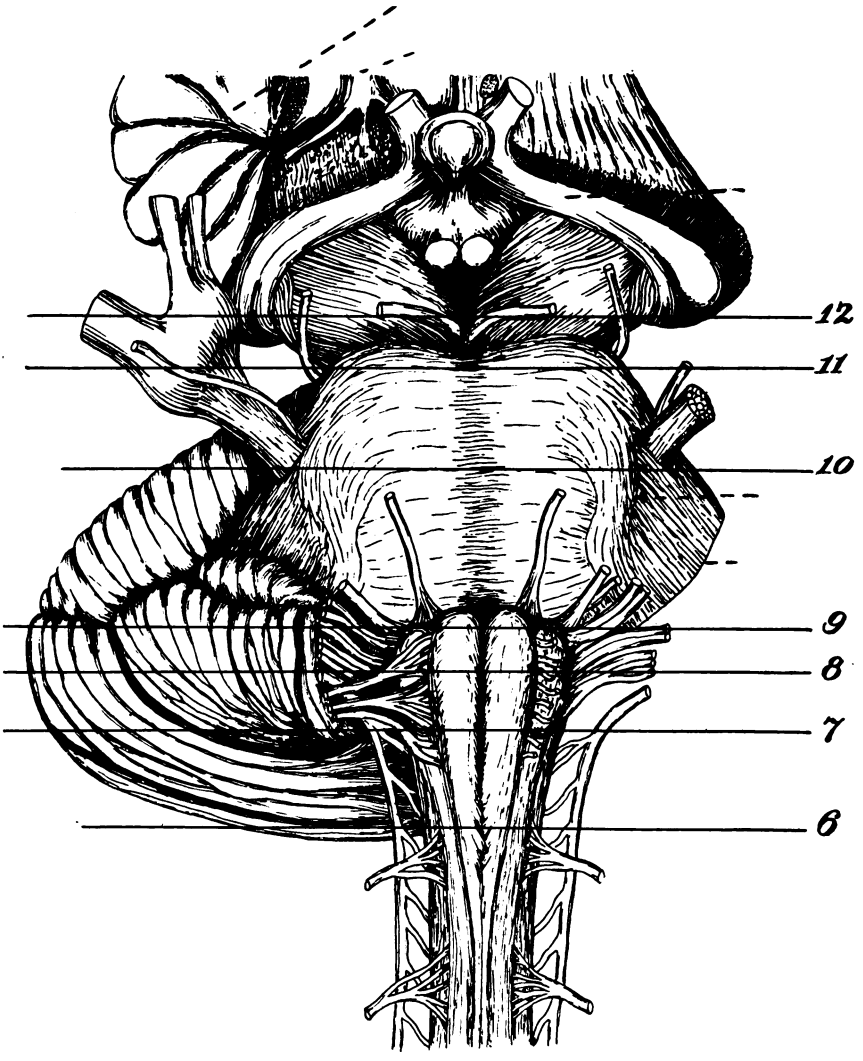

FIG. 23.—Showing the planes at which the sections through the Rhombencephalon and Mesencephalon are to be made (sections 6 to 12 inclusive).—*From Morris' Anatomy Modified.*

Pyramids, restiform body, inferior and accessory olivary nuclei, spinal tract of trigeminus with its nucleus, internal and external arcuate fibres, decussation of lemnisci, raphe, lemniscus and gray and white reticular formations; medial longitudinal fasciculus,

root filaments of hypoglossus and nucleus of hypoglossus; root filaments of vagus, nucleus ambiguus, tractus solitarius with its nucleus, nucleus of termination of the vagus and glossopharyngeus (nucleus alæ cinereæ), and the inferior or spinal part of the nucleus of the vestibular nerve.

What has become of the funiculi gracilis and cuneatus? Explain the changes in size, shape and position of the restiform body and state the origin, course and destination of the various axone systems composing it at this level. What is the origin, function and extent of the tractus solitarius? Relation of the nucleus of termination of the vagus to the commissural nucleus? Nature of the nucleus ambiguus and to what does it contribute fibres? What is the medial longitudinal fasciculus? Structure of the nucleus of the inferior olive and its relation to the arcuate fibres? What change has occurred in the position of Gower's tract and the descending fibres mingling with it? Character and analogy of the arcuate nucleus? Origin and character of the lining of the rhomboid fossa?

Section 9.—To pass close to the inferior border of the pons involving the tip of the superior extremity of the olive and slanting slightly upward, through the entering root of the acoustic nerve and onward through the cerebellum. Draw the stained section and complete the drawing by adding the portion of the cerebellum (not included in the stained section) from the anterior of the exposed surfaces of the cut specimen. Show and name especially the following:

Pyramids, lemniscus, raphe, medial longitudinal fasciculus, spinal tract of trigeminus with its nucleus, the arcuate nucleus, nucleus ambiguus, nucleus of tractus solitarius and root-fibres of glossopharyngeus; restiform body, cerebello-olivary (arcuate) fibres and olivary nucleus (or, if section includes inferior edge of pons, nucleus of superior olive and trapezoid body); acoustic medullary stria, acoustic tubercle, ventral nucleus and root of cochlear nerve; root of vestibular nerve, and medial, lateral and superior nuclei of vestibular nerve; nucleus and root-fibres of the abducens and nucleus and root-fibres of the facial nerve. Dentate nucleus, nucleus emboliformis and roof nucleus of the cerebellum; flocculus, inferior vermis, uvula of vermis, cerebellar gyri and sulci, cortical substance, medullary laminæ and medullary body of the cerebellum. Why are the pyramids larger here than in sections below this level? Which of the cerebellar peduncles are represented in the section? The relation between the inferior olivary nucleus and the cerebellum? Ascertain the relations between the lateral and superior nuclei of the vestibular nerve and the dentate and roof nuclei of the cerebellum, and state the functional significance of the relation.

What is the course of the acoustic fibres contained in the medullary striæ? How are the cochlear nerve, the nucleus of the superior olive and the trapezoid body related? Compare the course of the root-fibres of the facial nerve, from their nucleus of origin to their exit, with those of the abducens. Where do the fibres of the intermediate nerve arise? Why has the spinal tract of the trigeminus and its nucleus increased in size? How are the nuclei of the nerves of the two sides of the mid-line associated with each other?

Section 10.—To pass through the pons at the level of the entering roots of the trigeminus. Draw, giving special attention to the following:

Tegmental or dorsal part of the pons, and basilar part; brachium conjunctivum, brachium pontis, superficial and deep fibres of the pons, raphe, and gray substance or nuclei of the pons; pyramidal fasciculi, Gowers' tract and reticular formation; lemniscus, medial and lateral, and medial longitudinal fasciculus; the terminal nucleus and the motor nucleus (princeps), and the mesencephalic (descending) root of the trigeminus; anterior medullary velum, ligula cerebelli, fourth ventricle, central gray stratum, and locus caeruleus.

Explain the occurrence of a lateral and a medial lemniscus, and state the difference in their functions. Ascertain the course of the fibres of Gowers' tract and give the probable reason for their not coursing in the restiform body. What are the brachia conjunctiva? Explain the much greater sum area of pyramidal fibres here than in the levels below. Ascertain the course and termination of the frontal and temporal pontile paths. Explain the fact that the axones comprising the lemnisci are much more numerous than those composing the funiculi gracilis and cuneatus of the lower end of the medulla oblongata.

(b) *Sections of the mesencephalon.*

Section 11.—To snip the superior border of the pons and pass through the inferior colliculi of the corpora quadrigemina. Draws showing the following:

Lamina quadrigemina and tegmentum; pons fibres, peduncle of cerebrum, substantia nigra, lateral sulcus, brachia conjunctiva and their decussation, medial and lateral lemnisci and nucleus of lateral lemniscus; stratum zonale and nucleus of inferior colliculus; central gray stratum, mesencephalic (descending) root with motor nucleus of trigeminus, cerebral aqueduct, medial longitudinal fasciculus and nucleus and root fibres of the trochlear nerve.

State the course pursued by the root fibres of the trochlear nerve from their nucleus of origin to their exit, giving the locality and

detail of their decussation. Where are the pyramids? What portion of the cerebral peduncle will be occupied by the medial lemniscus in its further course? What is the significance of the nucleus of the lateral lemniscus and what relation does this nucleus and that of the inferior colliculus bear to the acoustic apparatus? What is the most probable path of the axons of longer course which associate the nuclei of the cranial nerves? What descending cerebral fibres are present other than those in the cerebral peduncles?

Section 12.—To pass through the region of the emergence of the oculomotor nerves and just anterior to the summits of the superior colliculi. The section will involve the pulvinar of the thalamus and the geniculate bodies which are parts of the prosencephalon. Draw, giving special attention to the following:

Basis of peduncle, substantia nigra, medial longitudinal fasciculus, lemniscus (medial), mesencephalic root of the trigeminus, central gray stratum and aqueduct of cerebrum; nucleus and root filaments of oculomotor nerve, nucleus and stratum zonale of superior colliculus, lateral geniculate body, pulvinar and optic tract; brachium quadrigeminum inferus, medial geniculate body and lateral reticular formation; interpeduncular ganglion, nucleus ruber, and decussation of the tegmentum. Pineal body, posterior commissure and stratum album profundum?

What is the relation of the nucleus ruber to the brachium conjunctivum and its decussation? Look up its relation to the fasciculus retroflenus of Meynert. To what is the name "substantia nigra" due? What is the nature, origin and extent of the mesencephalic root of the trigeminus and what can now be said of the central distribution of this nerve? Ascertain the arrangement of the three roots of the optic tract and their relation to the thalamus, lateral geniculate body, nucleus of the superior colliculus, and nucleus of the oculomotor nerve. Under compound microscope examine the nucleus of the oculomotor. What can be said of the size and arrangement of its cells? Consult texts as to its subdivisions and the functional significance of each. Locate the decussation of certain of the oculomotor fibres and the divisions of the nucleus from which they arise. By what bundle is the medial geniculate body connected with the acoustic apparatus? How may eye movements be associated with optic and acoustic impulses? Head movements? What is the origin, position in the section, and course of the "optic acoustic reflex path?" Look up the position and significance of the nucleus (ganglion) habenulæ. Nucleus of the posterior commissure?

(c) *Sections of the prosencephalon* (telencephalon and diencephalon).

Section 13.—To pass through the splenium of the corpus callosum and about the middle of the precuneus. The plane may slant toward the occipital pole. Draw, giving attention to the position and appearance of the following structures:

Cortical substance (cerebral cortex), white substance, radiation of the corpus callosum, occipito-thalamic (optic) radiation, and the tapetum; sulcus of the corpus callosum, gyrus and sulcus cinguli, precuneus, interparietal sulcus, lateral (Sylvian) fissure and the different gyri and the sulci separating them; the posterior horns of the lateral ventrical with its bulbus and the glomus of its chorioid plexus, the calcar avis and collateral eminence; collateral fissure, isthmus of gyrus fornicatus, hypocampal gyrus and fissure, and fusiform gyrus.

Identify the visual area and state the origin and distribution of the axones comprising the occipito-thalamic radiation. Of what center of cerebral activity is the precuneus a part? Is the olfactory area of the cortex represented in the section? How is the collateral eminence produced? Does the cortical substance vary in thickness? How, and where most?

Section 14.—To pass through the superior extremity of the posterior central gyrus and the posterior portion (pulvinar) of the thalamus. Draw, showing and naming the following:

Cortical gray substance, corona radiata, radiation of corpus callosum, corpus callosum, and fornix with tænia fornicis; inferior horn of lateral ventricle and central portion of same with chorioid tela of lateral ventricle, chorioid tela of third ventricle (velum interpositum), and internal cerebral veins; pulvinar of thalamus, cauda of caudate nucleus, cerebral peduncle, hypothalamic nucleus, internal capsule, claustrum, optic tract, hippocampal gyrus, hippocampal fissure the fimbria and fascia dentata hippocampi, and the gyrus and sulcus cinguli.

What is the corpus callosum? What is the relation of the optic tract to the pulvinar thalami? Why does the cauda of the caudate nucleus appear twice in the section? The relation of the cerebral peduncle to the internal capsule and corona radiata? Where is the posterior commissure of the cerebrum?

Section 15.—To pass through the mammillary body, the massa intermedia (middle commissure), and the anterior portion of the paracentral lobule. Draw, giving special attention to the following:

Cortical substance, medullary substance, radiation of corpus callosum, corpus callosum (trunk), corona radiata, internal capsule,

basis of peduncle, hypothalamic nucleus, ansa peduncularis, and optic tract; septum pellucidum, lateral ventricle (central portion and inferior horn), and the third ventricle; the thalamus with its massa intermedia, laminæ medullares, ventro-lateral, medial and anterior nuclei, its stratum zonale, tænia, stria medullaris, lamina affixa and stria terminalis thalami; collateral fissure, hippocampal gyrus, digitations of hippocampus, fornix (corpus), mammillary body, and fasciculus thalamo-mammillaris to anterior nucleus of thalamus; caudate nucleus, vena terminalis, lentiform nucleus composed of

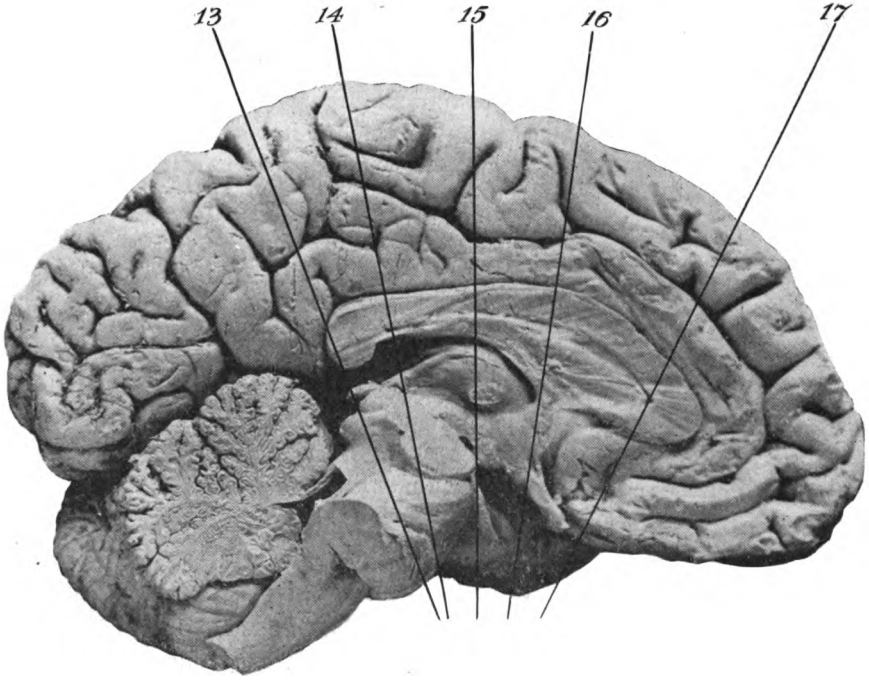

FIG. 24.—Showing the planes at which the sections of the Prosencephalon are to be made (sections 13 to 17).

putamen and globus pallidus, amygdaloid nucleus, inferior peduncle of thalamus, external capsule, claustrum and and insula with gyri insulæ. Attach the names to all the important gyri and sulci represented in the section, especially those of the temporal lobe.

What structures compose the corpus striatum? "Basal ganglia?" Where is the lemniscus and what is its relation to the hypothalamic nucleus and the ventro-lateral portion of the thalamus? Give the origin, course and distribution of the fornix and the relation of its fibres to the nuclei of the mammillary body. With what sense apparatus is it chiefly concerned? Where and what is the pedun-

culo-mammillary fasciculus? What is the position and significance of the hippocampal commissure or "lyre"? How many and what are the superior peduncles of the thalamus? What comprises the auditory area of the cerebral cortex and what are the paths of impulses to and from it and the rhombencephalic and mesencephalic connections of the acoustic nerve? What is the origin of the claustrum?

Section 16.—To pass through the optic chiasma, the anterior commissure and near the pars libera of the column of the fornix. Draw, especially showing the following:

Corpus callosum, column of fornix (?), anterior commissure, optic chiasma, anterior perforated substance, and uncus of hippocampal gyrus; septum pellucidum with its cavity (fifth ventricle), the anterior horn of the lateral ventricle and its chorioid plexus; caudate nucleus, internal capsule, and lentiform nucleus with its putamen, medullary lamina and globus pallidus; the gyri and sulci of the temporal lobe and insula.

In the section of the cerebrum removed in making section 16, note the triangular recess, the interventricular foramen, the depth of the third ventricle and the tuber cinereum. What cerebral lobes are connected by the anterior commissure? Where is the thalamus? What are the connections of the caudate and lentiform nuclei? In the optic chiasma, ascertain the position and relative amount of true optic fibres comprising the commissure, and the origin, position and siginficance of the inferior cerebral commissure (Gudden's commissure). Where is the superior cerebral commissure of Maynert?

Section 17.—To pass through the rostrum of the corpus callosum and the olfactory trigone. Draw, showing especially the following: Genu of corpus callosum, the septum pellucidum, rostrum of corpus callosum, lamina rostralis, sulcus corporis callosi, the two longitudinal striæ upon the corpus callosum, and the subcallosal gyrus; olfactory trigone (3 striæ evident?), the parolfactory area and parolfactory sulcus (?); the anterior horn of the lateral ventricle, caput of caudate nucleus, frontal part of internal capsule, lentiform nucleus (putamen only), external capsule, claustrum and insula.

Why does the lateral cerebral fissure occur twice in the section? What is the relation of the parolfactory area to the gyrus rectus and the olfactory trigone? What is the destination of each of the olfactory striæ? Note that the caudate and lentiform nuclei comprise a single ganglionic mass separated in part by the internal capsule.

4. Torn Preparation.—Lay out in their serial order all the drawings of the sections of the encephalon. Then take the cerebral hemisphere, detached in 2, (a), and, beginning at the sulcus cinguli, by means of the necessary tearing with the fingers, and aided from their appearances in the different sections, obtain a clear conception of the shape, course, interrelationships and connections of the following:

(a) The centrum semiovale, the varying thickness of the cortical gray substance (pallium), the underlying white substance by which the gray substance of neighboring gyri may be associated (fasciculi proprii), and that by which the cerebral lobes may be associated (association bundles).

(b) The corpus callosum, its splenium, trunk, genu and rostrum, with the medial and lateral longitudinal striæ upon it; the radiations of the corpus callosum, comprising an occipital portion (forceps major of the two hemispheres combined), a superior and lateral radiation to the parietal and temporal lobes, and a frontal radiation (forceps minor).

(c) The shape of the lateral ventricles, separated by the septum pellucidum, connected with the third ventricle and with each other by the interventricular foramen, and each consisting of an anterior horn, a central part, a posterior horn and an inferior horn; the central part and inferior horn containing the chorioid plexus.

(d) The anterior commissure connecting the temporal lobes and joined by commissural fibers from the medial olfactory stria.

(e) The hippocampus, the commissure of the hippocampus; the fornix, consisting of the crus, body and columns with pars libera and pars tecta; the mammillary body, the thalamo-mammillary fasciculus and the anterior tubercle of the thalamus.

(f) The shape of the caudate nucleus and its relation to the lentiform nucleus.

(g) The shape of the thalamus and its connection with the optic tract, the cerebral peduncle and the cerebral cortex. What is the relation of the stria medullaris thalami to the habenular commissure and habenular nuclei?

(h) The internal capsule consisting of frontal portion, genu, parietal portion and occipital portion, and continuous with the cerebral peduncle, pyramids and lemniscus below, and with the corona radiata to the cerebral cortex above. What is the relation of its genu to the stria terminalis thalami?

5. Microscopic structure.

(a) Under the compound microscope, examine a transverse (frontal) section taken at the level of the mammillary body and involving the

basis pedunculi, *thalamus* and *corpus striatum* only, and stained by the Weigert method and Upson's carmin. Sketch under an enlargement of about two diameters, indicating the abundance and arrangement of the cell-bodies and the occurrence and detailed arrangement of the white substance. Note the continuation between the medullary laminæ, stratum zonale and stria terminalis of the thalamus and the genu of the internal capsule. Explain the presence of the optic tract in the section. Which nuclei of the thalamus are represented and to which of these is the fasciculus thalamo-mammillaris distributed? What is the course and significance of the fasciculus pedunculo-mammillaris? Note the position of the hypothalamic nucleus and state its functional relation to the lemniscus and internal capsule. By what is the name of the lentiform nucleus suggested? Globus pallidus? What differences of shape and size are to be noted in the cell-bodies of the various nuclei in the section? By what paths and with what regions of the cerebral cortex are the nuclei of the corpus striatum connected?

(b) *Somesthetic Area of Cerebral Cortex.*—From the same block of cerebral cortex, taken from either the precentral or postcentral gyrus, two ventrical sections are furnished, one stained for the cell-bodies alone and the other by the Weigert method for the nerve-fibres. Under the same magnification, draw two vertical strips side by side, one strip from each section, showing the cell-strata and the arrangement of the fibres as follows:

Cell-strata:

(1) Superficial cell-stratum,
(2) Stratum of small pyramidal cells,
(3) Of large pyramidal cells,
(4) Of polymorphic cells, and
(5) Of fusiform cells.

Fibers:

(1) Plexus of molecular layer,
(2) Submolecular plexus,
(3) Great pyramidal plexus,
(4) Plexus of polymorphic cell stratum, and
(5) White substance continuous into fibræ propriæ (arcuatæ) and corona radiata.

What are the chief components and significance of the interstratal plexuses? Whence arise the different axones bearing impulses to the cells? Which cell-bodies give origin to the pyramids of the medulla oblongata and descending cerebrospinal fasciculi? Relative abundance of giant pyramidal cells in the section and significance of their being more abundant in the precentral than in the

postcentral gyrus? State briefly the general differences in the cell-strata of the cortex of the optic area, the auditory area, the uncus of the hippocampus and of the general structure of the so-called "association (psychic) centers."

(c) Make a careful drawing of a strip through the thickest (ventral) portion of a transverse section of the *olfactory bulb*, using either a successful Golgi or a Weigert preparation. Name the strata present and compare with the other regions of the cerebral cortex studied. Significance of the glomeruli? Which cells give origin to the olfactory tracts? Origin and more accepted destinations of the olfactory striæ? Existence of an olfactory ventricle? Origin of the latter and its persistence in comparative anatomy?

(d) Review the varieties of cell-bodies and the number and order of the cell-strata of the cerebellar cortex studied in the histology of the nervous system, ascertaining from texts the functional interrelation of the various cells and the variety and origin of the axones distributing impulses to them, and then construct a schematic representation of the architecture of the cerebellar cortex.

Considering the abundant connections of the vestibular nerve with the gray substance of the cerebellum, what significance may be inferred of the arrangement of the cerebellar gyri and the dendrites of the Purkinje cells? Other functions of the cerebellum?

III. RECONSTRUCTIONS.

Lay in their serial order all the 17 drawings made of the gross sections of the spinal cord, rhombencephalon and cerebrum, study them carefully with reference to the various structures appearing consecutively in them, and, with the aid of descriptive texts, construct diagrams of the central nervous system illustrating the following of the principal conduction pathways (systems of neurone chains).

A. Diagram of the Spinal Reflex and the Cerebrospinal Pathways.

1. Spinal reflex paths.

(a) Terminal corpuscle or "free termination" of peripheral process of T-fibre of spinal ganglion neurone.

(b) Afferent (sensory) axone in trunk of spinal nerve.

(c) Cell-body of origin in spinal ganglion.

(d) Dorsal root of spinal nerve.

(e) Bifurcation in spinal cord with ascending and descending branches giving off collaterals and terminating—

(1) Directly upon cell-bodies of ventral horn of same side in same level of spinal cord.

(2) Upon Golgi cell of type II, the axone of which terminates upon ventral horn cells.

(3) Upon ventral horn cells of opposite side, the terminal branch or callateral crossing the mid-line by way of either the anterior or posterior white commissure.

(4) Upon cell-body giving origin to axone of fasciculus proprius of same side, the branches of which axone terminate upon ventral horn cells in levels of spinal cord other than the level at which the dorsal root (afferent axone) enters.

(f) Ventral horn cells of same and opposite sides giving axones to ventral roots and trunks of spinal nerves directly to striated muscle-fibres or, indirectly, through the mediation of sympathetic neurones, to smooth muscle-fibres and gland cells.

2. Cerebrospinal path.

(a) Neurone of the first order—ascending:

(1) Terminal sensory corpuscle of peripheral process of T-fibre in trunk of spinal nerve.

(2) Cell-body with T-fibre in spinal ganglion.

(3) Dorsal root of spinal nerve.

(4) Bifurcation in spinal cord with both descending branch and collaterals above and below bifurcation terminating upon cell-bodies for spinal "reflex" paths as above.

(5) Ascending branch of bifurcation passing in fasciculus gracilis or fasciculus cuneatus to termination in medulla oblongata upon cell-bodies of—

(b) Neurone of second order—ascending:

(1) Cell-body in nucleus of fasciculus gracilis or of fasciculus cuneatus.

(2) Internal arcuate fibres.

(3) Decussation of lemniscus.

(4) Lemniscus of opposite side.

(5) Medial lemniscus to termination in diencephalon upon cell-bodies of—

(c) Neurone of the third order—ascending:

(1) Cell-body of hypothalamic nucleus or ventro-lateral nucleus of thalamus.

(2) Internal capsule, anterior part of its occipital portion.

(3) Corona radiata, fronto-parietal portion, to termination in somesthetic area of cerebral cortex (telencephalon) upon cell-bodies of—

(d) Descending system of neurones:

(1) Giant pyramidal cells of somesthetic area (pre- and post-central gyri, the former chiefly).

(2) Corona radiata, fronto-occipital portion.

(3) Internal capsule, genu and anterior part of occipital portion.

(4) Cerebral peduncle.

(5) Pyramid of medulla oblongata.
(6) Decussation of pyramids.
(7a) Lateral cerebrospinal fasciculus of opposite side of spinal cord, and
(7b) Ventral cerebrospinal fasciculus with gradual decussation to opposite side of spinal cord in cervical and upper thoracic segments, to terminate in ventral horn upon cell-bodies of—
(e) Efferent spinal neurones:
(1) Ventral horn cells.
(2) Ventral root of spinal nerve.
(3) Spinal nerve directly to termnation upon striated muscle-fibre, or, upon cell-body of sympathetic neurone, the axone of which terminates upon smooth muscle-fibre or gland cell.

B. Diagram of Principal Pathways of the Cranial Nerves Exclusive of those of Special Sense.

1. "Reflex" paths.

(a) Cell-bodies in ganglia of origin of sensory portions of vagus, glossopharyngeus, facial and trigeminus with peripheral arborizations and peripheral branches of T-fibres of same.
(b) Central branches of T-fibres of same into medulla oblongata where, bifurcated and unbifurcated, these branches send collaterals and terminal twigs to nuclei of termination (chiefly) of the respective cranial nerves, and some to nuclei of origin of motor cranial nerves and motor portions of mixed nerves.
(c) Cell-bodies of nuclei of termination give axones to motor nuclei of nerves of same side, and, through reticular formation and raphe, to motor nuclei of opposite side of same and of different levels, above and below, the axones to more distant nuclei coursing by way of medial longitudinal fasciculus.
(d) Axones from nuclei of origin of motor nerves and motor portions of mixed cranial nerves pass in these nerves to termination in respective tissues supplied, or upon cell-bodies of sympathetic neurones in respective chains concerned. Let the trigeminus, by way of its spinal tract, and the vagus and glossopharyngeus by way of the solitary tract, distribute impulses to the ventral horn cells giving origin to the ventral roots of upper cervical nerves.

2. Cerebral paths of cranial nerves.

(a) Ganglion cells of origin and peripheral and central branches of T-fibres of sensory portions of the vagus, glossopharyngeus, facial and trigeminus.
(b) Nuclei of terminations of central branches (bifurcated and unbifurcated) in medulla oblongata.

(c) Axones from respective nuclei of termination pass to reticular formation and medial lemniscus of same and (chiefly) of opposite side and ascend to their termination upon cells of hypothalmic nucleus and lateral nucleus of thalamus.

(d) Axones from cells of latter nuclei ascend in—

(1) Internal capsule, anterior part of occipital portion;

(2) Corona radiata, fronto-parietal portion,

(3) To terminate in somesthetic area of cerebral cortex.

(e) Axones of pyramidal cells of somesthetic area descend in—

(1) Corona radiata, fronto-parietal portion;

(2) Internal capsule, genu chiefly;

(3) Cerebral peduncle;

(4) "Accessory lemniscus" to terminate about,

(5) Cells of nuclei of origin of motor cranial nerves and of motor portions of mixed cranial nerves.

(f) Axones from these nuclei pass in the respective nerves to the muscles supplied, or terminate in sympathetic ganglia.

C. **Diagram of Spinal and Cerebral Pathways Involving the Cerebellum.**

1. Cerebello-spinal paths.

(a) Peripheral sensory fibres, spinal ganglia and dorsal roots of spinal nerves.

(b) Bifurcating dorsal root fibres in spinal cord give collaterals and terminal twigs to the cell-bodies of the nucleus dorsalis (Clark's column) and to cell-bodies (Stilling's nucleus) in the ventro-lateral neighborhood of the nucleus dorsalis.

(c1) Fibres from nucleus dorsalis ascend—

(1) As the cerebello-spinal fasciculus (direct cerebellar tract);

(2) Into restiform body (inferior cerebellar peduncle);

(3) Joined in medulla oblongata by external arcuate fibres (crossed and uncrossed) arising from nuclei of funiculus gracilis and funiculus cuneatus, which fibres make possible a second connection between sensory spinal neurones and the cerebellum;

(4) Joined in medulla oblongata by fibres arising in the nuclei of termination of sensory portions of vagus, glossopharyngeus, vestibularis and trigeminus.

(5) All these fibers pass through white substance of cerebellum to terminate about cell-bodies in dentate nucleus, nucleus fastigii (those from nucleus of vestibularis especially), and cerebellar cortex (vermis).

(c2) Axons from cells ventro-lateral to nucleus dorsalis ascend—

(1) As Gowers' tract in spinal cord;

(2) Through reticular formation of medulla oblongata and pons;

(3) Turn back in anterior medullary velum and brachium conjunctivum, enter white substance of cerebellum and pass to termination in cerebellar cortex (vermis chiefly) and dentate nucleus.

(d) Fibres from nucleus fastigii (chiefly), from dentate nucleus and from Purkinje cells of cerebellar cortex (vermis), descend in the intermediate and anterior marginal fasciculi to terminate about the ventral horn cells of the spinal cord.

Indirectly connecting the spinal cord with the cerebellum is the pathway arising from the nucleus ruber of the opposite side and decussating in the mesencephalon, and that arising from the nucleus of termination of the vestibular nerve of the same side, and descending in the intermediate fasciculus to be distributed to the gray substance of the spinal cord. Likewise, the olivary fasciculus of Helweg is an indirect cerebellar connection since the inferior olives are nuclei chiefly concerned with the cerebellum.

2. Cerebello-cerebral pathways.

(a) Fibres from the dentate nucleus and Purkinje cells of cerebellar cortex (vermis chiefly) ascend in brachium conjunctivum, cross to opposite side in decussation of brachia conjunctiva, and terminate in nucleus ruber and (chiefly) in lateral nucleus of thalamus.

(b) Fibres from these two nuclei (1) ascend in middle third of internal capsule and fronto-parietal part of corona radiata to terminate in somesthetic area of cerebral cortex and adjoining cortex of frontal lobe, and (2) pass by way of inferior peduncle of thalamus to cortex of temporal lobe (superior and middle gyri).

(c) Fibres from pyramidal cells of somesthetic area descend through corona radiata, internal capsule, and cerebral peduncle to nuclei of pons and to arcuate nuclei of same and opposite sides.

(d) Fibres from cortex of frontal lobe descend, as frontal pontile path, through frontal parts of corona radiata and internal capsule, and medial part of cerebral peduncle to nuclei of pons.

(e) Fibres from cortex of temporal lobe, as temporal pontile path, pass under lenticular nucleus into occipital portion of internal capsule and outer portion of cerebral peduncle, to nuclei of pons.

(f) Cells of nuclei of pons give origin to fibres which pass in brachium pontis to cortex of cerebellar hemisphere of the side opposite that of the cerebral hemisphere concerned.

D. Diagram of Pathways of the Auditory Apparatus.

1. *Vestibular portion.*

(a) Short peripheral processes from the cells of the vestibular ganglion pass to termination in the utricular and the three ampullar branches;

(b) Central processes of the cells of this ganglion (the vestibular nerve) enter medulla oblongata to terminate upon cells of the lateral,

medial and superior vestibular nuclei and the nucleus of the descending vestibular root.

(c) Fibres arising from the vestibular nuclei are distributed as follows:

(1) From the lateral and superior nuclei to the nucleus fastigii of the opposite side and to the dentate nucleus and cortex vermis (cerebellar connection).

(2) From the superior and medial nuclei, by way of the medial longitudinal fasciculus, to the nucleus of origin of the "eye-muscle" nerves of the same and opposite side.

(3) From the lateral nucleus and nucleus of the descending root, through the reticular formation, into the lateral funiculus of the spinal cord (spinal connection).

2. *Cochlear portion.*

(a) Short peripheral processes of bipolar cells of spiral ganglion terminate upon the neuro-epithelial cells of the organ of corti.

(b) Central processes of these cells combine to form the cochlear nerve and pass to medulla oblongata to terminate upon cells of ventral and dorsal nuclei of cochlear nerve.

(c) Fibres arising from cells of dorsal nucleus of cochlear nerve pass around outer side of restiform body and, as striæ medulares acustici, pass medianward in floor of fourth ventricle to mid-line, then ventralward into tegmentum where they decussate and join trapezoid body to lateral lemniscus of opposite side in which they pass to terminate in nucleus of inferior colliculus and medial geniculate body of that side.

(d) Fibres from ventral nucleus of cochlear nerve pass ventrally medianward, some to terminate in superior olivary nucleus of same side, others, through trapezoid body and lateral lemniscus, to terminate about cells of nucleus of superior olive, of lateral lemniscus, of medial geniculate body, and of inferior colliculus of opposite side.

(e) Fibres from superior olivary nuclei of both sides, and from nucleus of lateral lemniscus, terminate, some in nucleus of inferior colliculus, some in medial geniculate body, and probably some, uninterrupted, pass to termination in cortex of temporal lobe.

(f) Fibres from medial geniculate body and inferior colliculus pass into internal capsule and through temporal part of corona radiata to terminate in cortex of superior temporal gyrus (middle third chiefly).

(g) Certain fibres from superior olivary nucleus pass dorsalward to terminate upon cells of the nucleus of the abducens (peduncle of superior olive) and, by way of medial longitudinal fasciculus, to terminate in other nuclei of motor cranial nerves.

E. Diagram of Principal Pathways of the Optic Apparatus.

1. Short, "peripheral" processes of bipolar cells of retina terminate upon neuro-epithelial layer, the rods and cones, and their central processes terminate in conjunction with dendrites of ganglion cells of retina.

2. Fibres arising from ganglion cells form—

(a) Optic stratum of retina.

(b) Opitic nerve.

(c) Optic chiasma; fibres from nasal side of retina decussate in chiasma and fibres from lateral side of retina continue on same side.

(d) Optic tract, which terminates in three roots: One upon cells in pulvinar of thalamus; one in lateral geniculate body, and one passes under medial geniculate body to nucleus of superior colliculus.

3. Fibres from nucleus of superior colliculus pass ventrally to nuclei of oculomotor and trochlear nerves of same and opposite sides and, by way of medial longitudinal fasciculus, to nuclei of abducens, and these three nuclei send fibres to the muscles producing eye movements.

4. Fibres from cells of lateral geniculate body and pulvinar pass through occipital portion of internal capsule and occipito-thalamic radiation to terminate in cortex of occipital lobe (gyri bordering calcarine fissure, chiefly).

5. Fibres from visual area of cortex descend through occipito-thalamic radiation and internal capsule to terminate in nucleus of superior colliculus (occipito-mesencephalic fasciculus), and, by way of neurones of this nucleus, distribute cortical impulses to nuclei of nerves for eye movements.

6. Fibres from nucleus of superior colliculus and pulvinar enter medial longitudinal fasciculus of same and opposite sides and descend into ventral and lateral funiculi of spinal cord to terminate upon cells of its gray substance. Fibres from superior colliculus decussate in "optic-acoustic reflex path" in mesencephalon.

F. Diagram of Principal Pathways of the Olfactory Apparatus.

1. Short "peripheral" processes of bipolar cells in olfactory region of nasal epithelium terminate in "olfactory hairs," and centrally directed, non-medullated processes, the *olfactory nerve*, pass through lamina cribrosa of ethmoid bone to terminate in glomerular layer of olfactory bulb in conjunction with dendrites of mitral cells.

2. Fibres arising from mitral cells form olfactory tract which passes backward and divides into—

(a) Medial olfactory stria through which fibres pass to terminate (1) in parolfactory (Broca's) area, (2) in subcallosal gyrus, and (3), by way of anterior cerebral commissure, in olfactory bulb of opposite side.

(b) Intermediate olfactory stria which disappears in anterior perforated substance.

(c) Lateral olfactory stria which terminates (1) in anterior perforated substance and, chiefly, in uncus, in hippocampal gyrus proper, and in gyrus cinguli (olfactory area).

3. Fibres arising from cells in uncus and hippocampal gyrus comprise—

(a) The cingulum (in part), by which the cortex of the gyrus cinguli, subcallosal gyrus, and anterior perforated substance are associated.

(b) The hippocampal commissure (in part), through which they pass to terminate in the olfactory area of the opposite side.

(c) The fornix, which terminates (1) in small part upon cells in mammillary body of same and opposite side; (2) the remainder, accompanied by fibres arising in the mammillary body, passes in the thalamo-mammillary fasciculus to terminate in the anterior nuclei of the thalami.

4. Fibres arising in the mammillary body, and probably some from fornix direct, pass by way of the pedunculo-mammillary fasciculus into the cerebral peduncle and descend to terminate in the nuclei of the mesencephalon and medulla oblongata.

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THE ORGANS OF SPECIAL SENSE (ORGANA SENSUUM).

(TWELFTH PAPER.)

I. THE SKIN (INTEGUMENTUM COMMUNE), THE ORGAN OF TOUCH.

A. Macroscopic Features.

1. With dissecting lens examine the palm of the hand, the volar surface of the thumb or index finger, the dorsal surface of the hand and the skin of the fore-arm. What differences are to be noted in the different localities? How is the surface modified immediately about the insertion of the hairs (pili)? Pinch up the skin of the different localities and note apparent differences as to thickness.

2. Clean a side and dry it thoroughly, then carefully take an impression of the volar surface of the thumb upon it and examine under low magnification. Draw a small area showing cristæ and sulci cutis and the openings of the ducts of the sweat-glands (pori sudoriferi). Where do the latter open with reference to the former? Are the summits of the cristæ perfectly smooth?

What is the average area of the skin? Weight? Difference in thickness on dorsal and ventral surfaces of the body?

B. Microscopic.

1. From a stained vertical section of the skin of the volar surface of the finger, make a drawing under low power showing the peculiarities and inter-relations of the following components:

(a) Epidermis.

(1) Stratum germinativum (malpighian layer) composed of cells hexagonal in section and a basal layer of columnar cells resting upon a basement membrane.

(2) Stratum granulosum composed of two or three layers of lozenge-shaped cells containing deeply staining granules.

(3) Stratum lucidum, a thin transparent layer of cells not differentially straining.

(4) Stratum corneum, a thick layer in which the cytoplasm and nucleus has been exhaused in the production of horny envelopes and scales and through which the ducts of the sweat-glands pursue markedly spiral courses.

(b) Corium (dermis, cutis).

(1) Stratum papillare (tunica propria), sending numerous and variable finger-like papillæ into the stratum germinativum of the epidermis.

(2) Stratum reticulare, arbitrarily separated from stratum papillare, containing looser connective tissue, larger blood-vessels and tortuously running ducts of sweat-glands which pass to the peculiarly coiled bodies of their glands situated in the tela subcutanea and among the masses of adipose tissue, sections of blood-vessels and nerve twigs.

2. From the same section draw under high power (a) two or three cells from each of the strata of the epidermis, showing their shape, detailed structure and indicating their staining characters, and (b), draw a papilla corii covered by the basal layer of columnar epidermal cells and containing a Meissner's corpuscle (corpusculum tactus). What are the peculiarities of the cells of the stratum lucidum producing the appearance meriting the name? Note the apparent intercellular bridges in the Malpighian layer meriting the name "prickle cells." In which stratum may evidences of cell division be found? Trace the changes by which the cells of the stratum germinativum become transformed into the superficial scales of the stratum corneum. What is the structure of Meissner's corpuscle? Make a diagrammatic reconstruction of a sweat-gland with its entire duct. Look through the tela subcutanea for sections of Pacinian corpuscles (corpuscula lamellosa).

3. Compare the section from the volar surface of the finger with a similar section of the general skin of the body and enumerate the structural and quantitative differences between the two. Which layer of cells bears the pigment of the skin of the negro? Where are the papillæ corii more poorly developed and what can be said, in general, of the result of age upon their height? When may the tela subcutanea be called paniculus adiposus? Give two causes of the color of the skin of white races.

C. Appendages of the Skin.

1. The hair (pilus).

(a) Gently stroke the hairs of the back of the hand and short hairs of the scalp and note the extreme sensitiveness indicated. Note the various angles at which the hairs are inserted on the back of the hand and wrist. Gently pull a single hair of the wrist sufficiently to lift the epidermis about its insertion and carefully observe the region with a hand lens. Extract a hair, mount in glycerine, and examine the extracted end with the compound microscope, identifying its scapus, radix and bulbus. Appearance of peripheral end? From what regions of the surface of the body are hairs absent? What is the general law for the direction of the hairs of the body?

(b) From each of two stained sections of the human scalp, one passing longitudinally and the other transversely through the hairs and

hair follicles, make a drawing illustrating the structure and interrelations of the parts of a hair and its follicle as they appear cut in the two planes. Note that the follicle results from an invagination of the skin and that it therefore consists of an epidermal sheath and a sheath of connective tissue (corium), the layers of which two sheaths correspond to the strata of the epidermis and corium. In the drawing of the longitudinal section, include a sebaceous gland, showing its general structure and the relation of its duct to the follicle and the hair. Determine the significance and show the distinctive characters of the following components of hair and follicle:

- Hair
  - Papilla of hair
  - Medullary substance
  - Cortical substance
  - Hair-cuticle (outer hair membrane)
- Hair-follicle
  - Epidermal sheath
    - Inner root sheath
      - Cuticle of root sheath
      - Huxley's layer
      - Henle's layer
    - Outer root sheath
      - Layer of hexagonal cells
      - Layer of columnar cells
  - Connective-tissue sheath
    - Hyaline layer (glassy membrane)
    - Inner fibrous sheath
    - Outer fibrous sheath

Note that the medullary substance is not so well differentiated in the bulb as in the lower radix of the hair and that it is transformed into the lumen of the scapus; that the cortical substance consists of faintly outlined hexagonal cells in the bulb, elongated cells in the root, and flattened, non-nucleated scales in the scapus; and that the hair-cuticle consists of a layer of columnar cells in the bulb which gradually become inclined from the outside downward upon the cortical substance so that they overlie each other on the root, and, on the scapus, having lost their nuclei, they become superficial, imbricated scales, the outlines of which may be observed in surface views of the lower part of the scapus. Explain the differences in appearance of the layers of the hair-follicle in transverse sections at different levels. Compare the growth of the hair with the formation of the stratum corneum of the skin. What is the relation of the medullary substance to the layer of columnar cells of the outer root sheath and to the connective (mesodermal) tissue in the papilla of the hair? To what structure in the skin does the hyaline layer correspond? What cells bear the pigment of the hair? Examine the longitudinal sections of the follicles for an arrector pili muscle. Its two insertions and its arrangement with reference to the angle of insertion of the hair? How many sebaceous glands to each hair-follicle?

2. The nails (*ungues*).

(a) Macroscopic. Examine the nails of the hand and identify free margin, corpus, lunula, radix, lateral margin and vallum of the

nail. What is the relation of the stratum corneum of the skin to the eponychium and hyponychium? Explain the general color of the nail in position and the whitish appearance of the lunula.

(b) Pare a piece of nail from the free margin, place it in a small quantity of 20 per cent. potassium hydrate in a test tube and boil for a few minutes. Then place it in a drop of water on the slide, scrape or tease a portion, cover, tap the cover-glass, and examine the fragments under high power. What has been the action of the alkali upon the nail? Shape and arrangement of the cells? Nucleated? Compare with stratum corneum.

(c) From a longitudinal stained section passing vertically through a nail and involving that portion of the extremity of a finger lying dorsal to the third phalanx, make a drawing under low power showing the parts of the nail and its structure, position and intimate relation to the epidermis of the skin. Note that the corium of the skin forms the "nail bed" which, instead of sending papillæ corii into the epidermis, forms ridges parallel to the long axis of the nail which occupy furrows in the epidermis with cristæ between. Note further that the radix of the nail extends into a fold or duplication of the epidermis and thus, here, both the dorsal and concave surfaces are closely related to the stratum germinativum. Why does the radix diminish in thickness toward its termination? On the dorsal surface, what is the relation between the stratum corneum, the eponychium and the nail proper? What constitutes the matrix of the nail and what is its extent and relation to the lunula? Extent of the stratum granulosum? Where may papillæ corii be noted? To what stratum of the epidermis does the nail correspond? To what are occasional white areas in the body due? Describe the process of growth of the nail explaining the greater width of its free end.

D. Blood-supply of the Skin.

1. From a vertical section of injected skin, make a drawing illustrating the abundance and arrangement of its blood-supply, merely indicating the boundaries of the epidermis in outline. How is the stratum germinativum nourished? In what layer of the corium are the vessels largest? Of the general blood-supply of the body, what may be said of the proportion devoted to the skin?

E. Nerve Terminations of the Skin.

1. *Nerves of general sensibility.*

(a) From a gold chlorid or methylen-blue preparation of the epidermis, draw an area showing the so-called "*free terminations*" of axons in the stratum germinativum either upon the "prickle cells" or

among the cells of the basal, columnar layer. Note the repeated branching of the sensory fiber prior to termination. What is the relation of the telodendria to the protoplasm of the cells? Explain the cessation of the medullary sheaths.

(b) From a similar preparation involving longitudinal sections of hair-follicles (general skin of white rat or the large sensory hairs, vibrissæ, of the cat) study and illustrate the especially rich nerve-supply of the hair. Number of axones contributing to a single follicle? Note that the axone loses its medullary sheath upon entering the outer root sheath of the hair and then breaks up into a number of terminal twigs which encircle the epidermal sheath (plexus of Bonnet) below the sebaceous gland. Look for twigs terminating upon the hyaline layer and for twigs which penetrate this to terminate upon the epidermal cells. How are these stimulated? If the large sensory hairs are studied, note the peculiar blood sinus of the follicle. Its significance? If an arrector pili muscle is present, look for pilo-motor nerve terminations.

2. *Encapsulated terminations* (*corpuscle*) sub-epidermal.

(a) From the preparation used above or from any preparation showing it, draw under high power one of the tactile corpuscles of Meissner. Arrangement of the capsular tissue and the behavior of the terminating axone within it? Where does the medullary sheath cease? Position of the corpuscle as suited to its function?

(b) Make an illustration of a lamellated (Pacinian) corpuscle from one taken from the mesentery of the cat (methylen blue or osmic acid). Shape and size of the corpuscle? Of what tissue are the lamellæ formed? Are they nucleated? Their relation to the neurilemma of the axone? Behavior of the axone in terminating and its relation to the nucleated core of the corpuscle? As found in man, what is the position of this variety of corpuscle with reference to the skin?

Compare the corpuscles studied in (a) and (b) with illustrations or actual preparations of corneal corpuscles, genital corpuscles of Krause, and also with the corpuscles of Grandry and Herbst found in the modified skin of the duck's bill. What are the features of similarity existing throughout the series?

II. THE ORGAN OF TASTE.

A. Review the study of the macroscopic and microscopic features of the dorsum of the tongue made when considering the digestive apparatus (page 89). What of the position, appearance and relative abundance of the five general varieties of lingual papillæ? From personal experience, what may be assumed as to the abundance of the general sensory inervation of the lingual epithelium?

Which of the cranial nerves supply these afferent axones? What areas of the tongue and pharynx are most concerned in sensations of taste? Consider, physiologically, the intimate relations between gustatory and olfactory sensations.

B. From an ordinary stained section passing vertically through a vallate (circumvallate) papilla of the human (or monkey) tongue, make a drawing under low power, showing the whole papilla with its immediate surroundings and the position, shape and abundance of the *gustatory calyculi* (taste-buds). These structures are modifications of what? What in the lingual papilla correspond to the papillæ corii of the skin?

C. Under high power, draw a single longitudinally cut calyculus, showing the shapes and interrelationships of the varieties of cells composing it. What is the relation of the gustatory pore to the gustatory filaments and their relation to the neuro-epithelial cells? Significance of the arrangement?

D. From a gold chlorid or methylen-blue preparation of taste-buds (foliate papillæ of rabbit's tongue) make a drawing showing a taste-bud in outline and the entrance and form of termination of the gustatory axones. Which of the varieties of the epithelial cells is especially concerned?

Are taste-buds the only portions of oral epithelium concerned with sensations of taste? Discuss the adaptability of the vallate papilla to its assumed function. Which of the encapsulated nerve terminations does the taste-bud most resemble? To which cerebral gyri are gustatory impulses chiefly distributed? Give the most probable pathway and the number of neurones interposed in the chain by which the impulses reach this area.

III. THE OLFACTORY ORGAN.

A. *Macroscopic.*—Use the head of the sheep and pig fetus from which the brain has been removed, either fresh or that used and preserved in formalin when the preliminary studies of the central nervous system were made.

1. Note the two olfactory depressions near the mid-line in the anterior floor of the cranium, having the size and shape of the ventral surfaces of the olfactory bulbs, removed from them. Remove the dura mater and examine the floor of these depressions, the cribriform laminæ of the ethmoid bone, and note the numerous ethmoidal foramina through which the axones of the olfactory nerves pass to enter the olfactory bulbs. Variations in size of foramina? Peculiarity of olfactory axones?

2. Without injuring the orbits, remove the mid-portion of the frontal bone and cut away the nasal bones so as to expose the nasal cavity. What differences are observable between the respiratory and olfactory regions of the nasal epithelium? Remove a small piece of the olfactory epithelium, either from the mesial surface of the superior turbinated

bone or from the perpendicular plate of the ethmoid, place in 0.5 per cent. osmic acid four to twelve hours (if material has been preserved in formalin, wash well in water before placing in osmic acid), and then place in water for two days or more to macerate. What is the approximate area of the olfactory region in man? What device is accomplished by the turbinated bones (conchæ nasales)? What is the vomero-nasal (Jacobson's) organ?

B. *Microscopic.*

1. From an ordinary stained vertical section of the epithelium of the olfactory region, draw a narrow strip under medium magnification, showing the nature of the epithelium and the tela submucosa. How many layers of nuclei? Differences of epithelium from that of respiratory region? Where is the pigment situated to which is due, in the fresh, the difference in color between the two regions? How do the nuclei of the olfactory cells differ from those of the sustentacular cells? Explain "olfactory hairs." Abundance, nature and significance of glands of Bowman?

2. Tease on the slide a bit of the epithelium put aside in A–2, above, to macerate, mount in water and examine? Sketch some of the isolated sustentacular cells and one or two olfactory cells. Varieties of the former and distinguishing peculiarities of the latter?

3. From vertical sections of olfactory epithelium (fetal pig or newborn rat or rabbit), stained by the Golgi method, make a drawing, showing the shape of the olfactory cell, its relation to the thickness of the mucous membrane and the origin and course of the axone. Does the axone branch?

3. From Golgi preparations of the olfactory bulb, sectioned vertical to its ventral surface, construct a drawing showing its different strata, the varieties of its cell-bodies and their relation to the olfactory tracts. Significance of the glomeruli as related to the olfactory cells and mitral cells? What gyri comprise the cortical area of smell? Beginning with the olfactory epithelium, name in their functional order the different pathways and masses of gray substance comprising the olfactory apparatus.

The studies called for in 2 and 3 may both be made from a Golgi section passing vertically through the upper and anterior part of the head of a fetal rat or rabbit.

IV. THE ORGAN OF HEARING.

A. **External ear.**

1. With the aid of an atlas study the position and conformation of the living *auricle* (pinna) with reference to its function. Sketch its lateral aspect naming the principal parts. In the ears of different individuals,

note the variations in the lobule, the tragus, antitragus and crus of the helix. Occurrence of a supratragal tubercle? Note that the sulci and fossæ are roughly continuous with each other and into the external acoustic meatus. How is the opening (cartilaginous portion) of the latter guarded against foreign particles? What is the position, morphology, origin and function of the ceruminous glands? Of what variety is the cartilage of the auricle and that of the external meatus?

2. *The tympanic membrane.*

(a) From descriptive texts or atlas determine the shape and environment of the membrane and the angle at which it is placed with reference to the external acoustic meatus. Carefully dissect the head of the fetal pig (specimen used in preliminary study of the central nervous system) so as to expose the membrane. Note its variations in thickness, its tense and flaccid regions, its border (limbus) and the attachment of the manubrium of the malleus (umbo and malleolar prominence). What predominating arrangement of the fibrous structure of the membrane is evident? Remove a portion, mount in water and examine under low power. What is the difference of the two sides?

(b) From a vertical stained section of the tympanic membrane taken away from the attachment of the manubrium, make a drawing under high power illustrating its three layers: (1) The cutaneous layer, continuous with the stratified squamous epithelium lining the external meatus and with no papillæ corii. How many layers of cells? Nature of stratum corneum? (2) The fibrous layer (lamina propria) consisting of an inner circular stratum of connective tissue fibers and an outer stratum radiating from the attachment of the manubrium of the malleus. Blood-vessels? (3) The mucous layer consisting of a layer of simple epithelium resting upon a basement membrane which is intimately connected with the circular fibrous stratum. From which germ layer is this epithelium derived?

B. **The Middle Ear** (*tympanum, tympanic cavity*).

1. Become familiar with the position and size of the cavity and with the parts and relationship of the three auditory ossicles. Is the arrangement of the ossicles such that the vibrations of the tympanic membrane are magnified as transmitted to the membrane closing the fenestra tympani? Attachments and action of the tensor tympani muscle? Origin, function and course of the chorda tympani? Nature of the lining of the tympanic cavity and its variation in the region of the opening of the Eustachian or auditory tube? What is the structure of the wall of this tube and for what purpose and with what does it connect the tympanic cavity?

2. Remove the *osseous labyrinth* from the head of the fetal pig used above. (In pig fetuses at term or shortly after birth, the labyrinths have not become fused to the surrounding petrosal portion of the temporal bone and may be "shelled out" without difficulty.) Insert a needle between the crura of the *stapes* and remove it from its position over the fenestra vestibuli (ovalis). Return the labyrinth to formalin solution and place the stapes in a watch glass of water (or dehydrate, clear, and mount in a cell slide) and draw under low power showing capitulum, anterior and posterior crus, basis, and obturator membrane of stapes. Is this membrane perforated naturally? Shape and peculiarity of basis of stapes? Attachment and action of stapedius muscle?

C. **The Inner Ear** (*the labyrinth*).

1. Place the *osseous labyrinth* (removed above) in a watch glass with water enough to cover it, and, under dissecting microscope, make outline drawings of both the mesial or posterior and the lateral or anterior aspects, showing and naming the three semicircular canals with their ampullæ, the vestibule with fenestra vestibuli and fenestra cochleæ, and cochlea with its cupola and base. It is desirable to dissect away some of the bone to better expose the semicircular canals and vestibule. Note the acoustic nerve divided into its vestibular and cochlear branches. By reference to texts and illustrations determine the position and significance of the sacculus, utriculus, endolymphatic duct, the communications of the semicircular canals with the vestibule, and the three maculæ cribrosæ (perforated spots). Return the osseus labyrinth to the formalin solution.

2. *The membranous labyrinth.*

(a) From a stained section of the entire labyrinth involving a vertical axial section of the cochlea (fixed for three days in Zenker's fluid or for twenty-four hours in Perenyi's fluid, either of which fluids decalcifies while fixing), draw under low power showing the interrelations and naming the following structures of the cochlea:
The cochlear nerve, spiral ganglion, osseous spiral lamina, basilar lamina (lamina spiralis membranacea), basilar crest, spiral ligament, spiral prominence, spiral organ (organ of Corti), spiral limbus, spiral sulcus, vestibular lip (with Huschke's teeth), tectorial (Corti's) membrane, vestibular (Reissner's) membrane, scala vestibuli, duct of cochlea, scala tympani, and spiral canal (tunnel of Corti). What is the modiolus? Look for hamulus of spiral lamina. What is the helicotrema? Its functional significance? Derivation and general course of the blood-supply of the cochlea?

(b) Make a drawing under high power including the spiral limbus and sulcus, the tectorial membrane, the basilar lamina, and

showing in detail the spiral organ (organ of Corti). What is the tympanic lip? What is the difference between the cells of Claudius, cells of Hensen, and Deiters' cells? Nature, functional significance and difference in number of outer and inner hair-cells? What is the linear arrangement of the pillars (rods) of Corti? Of the pairs of pillars, which is the longer and more slender and where are their nuclei situated? What is the origin, structure and functional significance of the tectorial membrane? From its variations as seen in sections in the different turns of the cochlea, what can be said of the shape of this membrane? Note that the basilar lamina consists of a tympanic, cellular lamina which contains the tortuous vas spirale, and of an upper, dense connective tissue lamina, the *basilar membrane.* What theoretical significance has been attached to the latter and what facts of structure are urged against the theory? What is the structure of Reissner's membrane? What variety of nerve cell-body comprises the spiral ganglion? How do the terminal twigs of the peripheral axones of these reach the outer hair-cells?

(c) Gently crush the osseous labyrinth, studied above, place in watch glass of water under the dissecting microscope and, with teasing needles and fine-pointed forceps, carefully remove the bits of bone so as to expose the membranous labyrinth. Work over a black surface. How many turns in the cochlea of the pig? Transfer to clean water and carefully remove (tear off with the forceps) the outer portion of the wall of the scala vestibuli and identify cupular cecum, helicotremma, Reissner's membrane, the tectorial membrane (very fragile), the vestibular lip of the spiral lamina, and the basilar membrane. Remove bits of the spiral structures upon a slide and examine with compound microscope, using low light. Note "Huschke's teeth" as seen on the flat; the structure of the basilar membrane and the course of the vas spinale below it; the ease with which Reissner's membrane is removed and the course of the blood-vessels within it; and the fact that the tectorial membrane easily floats free from its attachment when disturbed. If possible, mount a portion of this membrane, basal surface upward, and examine under high power. Its fibrous structure, supported in a very flexible glutinous substance, may be observed and the peculiar arrangement of the fibres producing the appearance of its three zones, and Hensen's line, which lies over the interlocking phalanges of the pillars of Corti, may be identified. What is the relation of the ascending scala vestibuli to the descending scala tympani with reference to wave motion transferred to the fluid in them?

(d) Name the four branches of the vestibular division of the acoustic nerve and the localities of their termination. Where are the cells of origin of the saccular (a branch of the cochlear nerve) and of the

posterior ampullar nerves? To what in the cochlea do the hairs of the maculæ accusticæ of the utriculus and sacculus, and those of the cristæ of the ampullæ correspond? The otolith membranes and the cupulæ? Significance of otoliths? In the preparation used in (a) and (b) above, identify sections of the semicircular canals and of the sacculus or utriculus. In a section of a semicircular canal, away from the ampulla, note the relatively small *endolymph canal* (semicircular duct) and the much larger perilymph-space separated by connective tissue partitions (ligaments), all the spaces being lined by a simple, flat epithelium. Difference of origin of the epithelium of the endolymph canal? Name and position of the duct by which the original continuity of the entire membranous labyrinth is retained?

Sketch a section of a semicircular canal, naming locality and parts represented.

3. What cerebral gyri and what portions of them comprise the cortical area of hearing? With what divisions of the encephalon are the central connections of the vestibular nerve peculiarly concerned? Review the location and connections of the nuclei of termination of the acoustic nerve and, beginning with the organ of Corti and the maculæ and cristæ acousticæ, construct a diagram illustrating the variety, course and connections of the neurones of the acoustic apparatus.

V. THE ORGAN OF VISION.

A. Macroscopic.

1. With the aid of a mirror, sketch the eye in position, showing the supercilium (eyebrow) with the varying directions of the hairs composing it; the superior and inferior palpebræ with their cilia (eyelashes), their commissures and anterior and posterior limbs; the medial and lateral anguli oculi; the coruncula and papilla lacrymalis (opening of duct of lacrymal gland); the cornea with the iris and pupil showing through it, and the sclera. Note the annuli and zones of the iris. Allow changes in the intensity of the light entering the eye and note changes in the diameter of the pupil produced by the accomodative activity of the ciliary muscles. Retract the inferior palpebra and observe that the conjunctiva bulbi is continuous with the conjunctiva palpebrarum. Note the superficial blood-supply of the bulb.

2. Carefully dissect out the bulbus oculi of the sheep (from the head preserved in formalin when making the preliminary study of the brain), retaining a portion of the optic nerve and the eye-moving muscles attached to the bulb. Determine the shape, relative size and locality of attachment of the six extrinsic muscles and give the name, function and innervation of each. Note the occurrence and peculiarity of arrange-

ment of the M. retractor bulbi possessed by the sheep. What distinguishes the superior oblique muscle? Size, position and appearance of the lacrymal gland? Dissect so as to determine the relation of the cranial dura and pia mater to the optic nerve.

3. With razor bisect the bulb in the horizontal meridian of the optic nerve. Place in water and identify the following:

(a) *Anterior camera oculi* (anterior chamber) containing the aqueous body (humor).

(b) *The cystalline lens.*—Note its position and the difference in curvature of its anterior and posterior surfaces. Action of the formalin upon it?

(c) *The posterior chamber* containing the vitreous body (humor). Difference between this and aqueous body? Note course of hyaloid canal and hyaloid artery. Where is the posterior camera?

(d) *The three tunics of the eye.*

(1) The *fibrous or external tunic* forming the sclera and the cornea. Difference in appearance of the two parts and the action of formalin upon the latter? Determine the existence of the anterior corneal epithelium (conjunctivum). Tease a bit of the cornea and examine, mounted in water, for the direction of its structural arrangement and the presence of nuclei.

(2) The *vascular or middle tunic* comprising the chorioidea, the ciliary body and the iris. Of the chorioidea, note the lamina vasculosa, containing numerous blood-vessels covered with pigment, and the thin lamina basilaris. Of the ciliary body, distinguish the ciliary folds and processes (corona ciliaris), the orbiculus ciliaris, and the ciliary zone (zone of Zinn) consisting of zonular fibres attaching the ciliary body to the capsule of the crystalline lens (suspensory ligament). Of the iris, a continuation of the chorioidea and ciliary body, note the stroma of the iris, consisting of abundant connective tissue and radially arranged blood-vessels, and the posterior pigment layer of the iris.

(3) The *retina or internal tunic*, the thinnest of the tunics and distinguished from the middle one by its whiteness. It lies loosely except for its attachment to the ciliary body and its continuation into the optic nerve, and consists of three portions. Distinguish its optic portion and its ciliary portion (pars ciliaris retinæ), the boundary between the two portions appearing as a zig-zag line, the ora serrata. The ciliary portion extends from the ora serrata to the outer border of the iris. The third or iridic portion of the retina underlies the iris, extending from its outer or ciliary border to its pupillary border and, therefore, forms the posterior or pigment layer of the iris mentioned above. Remove a bit of the posterior portion of the pigment layer of the retina,

continuous with the ciliary and iridic portions, mount it in water and examine, distinguishing it from the adherent pigment cells of the chorioidea. Peculiarities? Note the papilla of the optic nerve with its central depression (excavation).

From which of the germ layers are each of the three tunics of the eye derived? From its development, to what does the retina correspond? To which encephalic meninges do the sclera and chorioidea correspond? Origin and localities of entrance of the blood-vessels of the eye-bulb? Examine the optic nerve for the central vessels of the retina.

(e) With dissecting microscope examine the crystalline lens under water over a black surface. What is the apparent arrangement of its structure? Tease the lens, identifying its capsule, cortical substance and nucleus. What is the shape of the lens-fibres and how do they run? Nature of cement substance? Why does the lens tend to break up into concentric lammellæ? Origin of the lens?

B. Microscopic.

1. Under low power, draw a stained horizontal section of an entire bulbus oculi (human, monkey or dog, fixed in Perenyi's fluid for softening the lens and embedded in celloidin). Show the arrangement of and name the structures identified above. In addition, show that the muscles of the ciliary body and iris are, in each, arranged into a radial (meridianal) system and a circular system. Explain the function of the fibres belonging to each system. Origin of the motor axones supplying the ciliary muscles and the radial and sphincter pupillæ of the iris? Note the cessation of the optic portion of the retina at the ora serrata, and explain the function of the zonalar fibres (ciliary zonule) which pass from the ciliary body and are attached upon the capsule of the crystalline lens. Indicate the epithelium of the lens, the arrangement of the lens-fibres and the locality in which the fibres possess nuclei. Nature of the lens capsule? Is there both an anterior and a posterior corneal epithelium? Identify other laminæ of the cornea, the venous sinus of the sclera (the canal of Schlemm), and the ciliary blood-vessels. What is the difference between the cells of the chorioidea and those of the pigment layer of the retina? Look for the lamina chorio-capillaris and the lamina basilis of the chorioidea. What is the difference between the optic axis of the bulb and the visual axis? Where is the macula lutea (yellow spot) with its fovea centralis, and from its situation what may be inferred concerning it?

2. From a stained, thin, vertical section of the optic portion of the retina (human or dog, removed separately and sectioned in paraffin), make a careful drawing under high power of a small segment showing and

naming, from the outer inward, in their order and relationship, the eleven strata into which the three actual layers of functional elements are divided, as follows:

(a) Pigment layer of the retina (pigmented epithelium).

(b) The neuro-epithelial layer (*radio-sensitive or visual cells*)
- (1) Layer of rods and cones.
- (2) External limiting membrane.
- (3) Outer nuclear layer.
- (4) Henle's fibre layer.

(c) Ganglionic layer (*cerebral layer*)
- (1) Outer reticular (molecular) layer.
- (2) Outer ganglionic (inner nuclear) layer.
- (3) Inner reticular (molecular) layer.
- (4) Inner ganglionic (ganglion cell) layer.
- (5) Nerve-fibre (optic nerve) layer.
- (6) Internal limiting membrane.

What are the rods and cones cytologically? Number and appearance of the segments into which they may be divided? Arrangement of the nuclei to which they belong? Numerical relation of rods to cones? What is the external limiting membrane? What is the source of rhodopsin or "visual purple" and upon which elements does it act? Changes in nerve-fibre layer in passing from ora serrata to papilla of optic nerve (see section used above), and its relation to inner ganglionic layer and to optic nerve? What constitutes the internal limiting membrane? What differences are to be noted in the nuclei of the different layers? Compare the drawing with a section passing through the fovea centralis (demonstration preparation if section used does not involve fovea). Note the changes in the different strata producing the fovea centralis and explain the reason for the name "*macula lutea.*" Significance of the peculiar character of the neuro-epithelial layer in region of fovea? Behavior of the nerve-fibre layer in the region of the fovea?

3. With the aid of Golgi preparations of the retina and descriptive texts, construct a drawing showing the significance and intimate relationship of the retinal elements composing the layers designated above. Include one or two of the radial masses of the supporting tissue (fibres of Müller) of the retina, showing their peculiarities of form, the retinal layers through which they extend, the layer to which their nuclei belong, and their relation to the external and internal limiting membranes. What comprises the outer and inner reticular layers? Compare the "bipolar cells" (nuclei of which form outer ganglionic or inner nuclear layer) with the neurones of a spinal ganglion, and give reasons for their being called the "*ganglion retinæ.*" Indicate the position, shape and functional significance of two or three varieties each of *amacrine cells* and *horizontal cells*. Why are they so-called? To what cells in nerve ganglia and in the cerebral and cerebellar cortex do they correspond? Examine the drawing made in 2, above, for nuclei probably belonging to amacrine

cells and so label them. Also include in the present drawing a centrifugal axone (entering the retina from the direction of the central nervous system), showing its course and the retinal layer in which it terminates. Give physiological evidence for the existence of such axones.

4. Construct a diagram illustrating the varieties and arrangement of the retinal cells and the neurones, cerebral and retinal, interposed in the visual apparatus, naming the different portions of the pathways and the nuclei involved, and indicating the cerebral gyri comprising the cortical area of vision. In what animals in general is there a total decussation of optic fibres in the chiasma? Which fibres decussate in man? What axones form the inferior cerebral (Gudden's) commissure? How are eye movements associated with visual impulses?

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SECTION III.

THE METHODS OF PREPARATION EMPLOYED.

To accomplish the anatomical studies suggested in Section II, the student will not have time to apply the methods involved in the preparation of his material other than those few for which directions are given in that section. It is considered neither advisable nor wise that he should spend time, which should be devoted to the real subjects of the course, upon the routine methods of making sections and staining. A far more economic arrangement is that the sections be prepared for the whole class by one man especially experienced in this work, and such an arrangement is expected here. Some familiarity with the general processes of fixing, embedding, sectioning and staining is assumed to have been attained by the student in his high school work and in courses taken prerequisite to this. Such familiarity will enable him to follow the easily available directions for any of the special and more complicated methods should occasion arise later for him to employ them.

Detailed descriptions of the various methods employed in making the preparations suggested in this Laboratory Guide are not deemed necessary from the fact that they are given in all the descriptive texts of histology, some one of which must be used in conjunction with this guide. For a greater variety of methods, and modifications of the methods used here, the student must, at need, consult the reference works devoted to microscopical technic, such as Lee's Microtomists' Vade-Mecum, Guyer's Animal Micrology, Hardesty's Neurological Technic, or, for example, the numbers of the Zestschrift für wissenschaftliche Mikroscopie and the Zentralblatt für Normale Anatomie und Microtechnik. It may at times be necessary to refer to the original paper of the author who has devised a method or a modification of a method for obtaining special results.

In order that the student, and the assistant who prepares the material for the course of study outlined, may more easily see what methods are called for, they are enumerated below. All of the fluids are in common use and their formulæ may be found in the reference books or in the papers cited.

1. *The general method*, that by which most of the sections are prepared, consists in fixing the tissue in Gilson's or, preferably, Zenker's fluid, washing as required; embedding in celloidin, staining the sections with Delafield's hematoxylin, decolorizing with acid alcohol, couterstaining with 0.1 per cent. congo red, dehydrating with graded alcohols, distributing the sections in the students' section dishes, which contain creasote or any good clearing mixture for celloidin sections, where they are cleared and from which they are placed on the slides by the students, the surplus clearing fluid blotted up with filter paper

and the sections mounted in balsam. Congo red as a counterstain is preferable to the commonly used eosin because it brings out cell boundaries and delicate reticula better, and also differentially stains certain gland ducts, gastric cells, etc.

2. *Van Giesen's Method.*—Celloidin sections are prepared as above, stained with Delafield's hematoxylin, and simultaneously decolorized and counterstained with Van Giesen's picric-acid-fuchsin mixture, washed and dehydrated in alcohol, and distributed to the students as above.

3. For *cell division.*—(a) Vegetable tissue is fixed in Flemming's fluid, washed as required after that fluid, embedded in paraffin, sectioned thin, sections fixed on slide, paraffin removed with xylol, sections carried through graded alcohols, to 50 per cent. alcohol, and stained 12 to 24 hours in a mixture of equal parts saturated alcoholic solution of safranin and anilin water. Sections are then decolorized in anilin alcohol, rinsed in 50 per cent. alcohol, stained 2 to 4 hours in a saturated aqueous solution of gentian violet, rinsed in anilin alcohol, dehydrated (a few seconds) in absolute alcohol, differentiated with clove oil, dipped through xylol, and mounted in balsam. The necessary times for decolorization and differentiation must be determined by examination under the microscope.

(b) Animal tissue is fixed in either saturated corrosive sublimate and washed as required, or fixed in Van Gehuchten's (Carnoy's) fluid, embedded in paraffin, sectioned thin, sections fixed on slide, paraffin removed, sections treated with alcohols, stained by Heidenhein's Iron-hematoxylin method, counterstained with orange G. or weak fuchsin, washed, dehydrated, cleared in xylol and mounted in balsam. They may be stained with Delafield's hematoxylin and lightly counterstained with congo red or eosin.

4. *Digestion* for Reticular Tissue.—Small blocks of fresh spleen or lymph-gland may be fixed in 96 per cent. alcohol, Van Gehuchten's fluid or Zenker's fluid, washed well with water and subjected to the action of pancreatin dissolved in weak sodium bicarbonate, in a thermostat as described by Flint (Johns Hopkins Hosp. Bull., vol. 13, 1902). Afterward the block of tissue is carefully washed, embedded in paraffin, and sections stained with acid fuchsin or anilin blue. Or, thin free hand sections of the fixed blocks may be digested in the pancreatin solution, washed, stained, dehydrated, cleared, and mounted. The latter is the more rapid method but is too coarse for delicate frameworks.

5. *Injections.*—For the finer blood-supply of tissue and organs, a good carmin-gelatin injection mass is preferable. It is employed in the usual way with animals which have been chloroformed long enough for relaxation of the tissues to take place, the injection long continued under low pressure, the whole hardened and cooled in alcohol for several hours and then the parts desired removed and further hardened and preserved in alcohol. The precautions followed by Walker in making the injection mass will give excellent results (Am. Jour. Anat., vol. 5, No. 1, p. 73, 1905). For the lymphatics, an injection mass, more fluid than the gelatin mass, is necessary. An aqueous solution of Berlin blue is often used. Instead the haphazard, commonly used,

puncture method of injecting lymphatics, in most cases it will be found more expedient to use the "needle and clamp device" described by Prof. W. S. Miller (Johns Hopkins Hosp. Bull., vol. 16, No. 173, 1905).

6. *The Golgi method* is employed for three purposes: (1) For the demonstration of neuroglia and of the nervous elements in both their central and peripheral relations; (2) For certain of the ducts of the gastric glands, and (3) for the bile capillaries. For fresh tissue, "the rapid method" is used in which the fixing qualities of the mordanting fluid, 3.5 per cent. potassium bichromate, are aided by the addition of osmic acid. Slight modifications are practiced in applying the method for the different purposes. Bile capillaries usually require less time in both the bichromate and the silver nitrate solutions than is required for nervous tissues. From the silver bath, to obtain more even sections, the blocks of tissue are incisted in celloidin before cutting. Small blocks of formalin preserved tissue may be subjected to the 3.5 per cent. bichromate, without the addition of osmic acid, and then treated with the 0.75 per cent. sliver nitrate solution in the usual way. When successful, the preparations from formalin tissue are most satisfactory in that the pictures of the elements lie in a much more transparent background, and in that the precipitate of reduced silver chromate seems less liable to break up and render the preparations diffuse.

7. *The Benda method* is employed for the detailed study of the neuroglia. The alizarin sulphate in this method gives an excellent background for the bright blue neuroglia fibres and nuclei and stains the endoplasm of the cells as well. The procedure followed by Huber (Am. Jour. Anat., vol. 1, No. 1, 1901) is recommended. The Benda method is also used for demonstrating the framework of the medullary sheaths of axones.

8. *Weigert's method for elastic tissue* is applied to material fixed in Zenker's fluid. The fuchsin-resorcin solutin of Weigert is applied to sections, preferably paraffin sections, for from 1 to 2 hours. The sections are then washed in 95 per cent. alcohol, differentiated in a 0.5 per cent. solution of picric acid in 95 per cent. alcohol, washed and further dehydrated in absolute alcohol, cleared in xylol and mounted.

9. *Orcein* for elastic tissue fibres is applied to either paraffin or celloidin sections, the latter giving somewhat better results. The tissue may have been fixed in Zenker's or Van Gehuchten's fluid, or most any of the efficient fixing fluids. Sections are placed in a watch glass containing sufficient to well cover them of a 1 per cent. solution of orcein in absolute alcohol to which there is added 1 per cent. of hydrochloric acid. The watch glass is either put in a warm oven till the stain evaporates to viscosity (15 to 20 minutes), or is covered and put aside allowing the stain to act for 24 hours. The sections are then washed and dehydrated in 95 per cent. alcohol, cleared and mounted. If further decolorization and differentiation is necessary, 95 per cent. alcohol containing 0.3 per cent. to 0.5 per cent. picric acid may be used, then wash in pure 95 per cent. alcohol, clear and mount as before.

10. *Mallory's method* for white fibrous tissue is used with paraffin sections of material fixed in Zenker's fluid. After the parffin is removed from the sections, fixed on the slide, the sections are carried through the graded alcohols to water, stained 5 to 10 minutes in 0.1 per cent. aqueous acid fuchsin, washed in water, placed 1 to 2 minutes in a 1 per cent. aqueous solution of phosphomolybdic acid, then washed in 2 or 3 changes of water, and subjected for from 1 to 10 minutes to an aqueous solution made by adding to 100 c.c. of boiling water, 1 gram of anilin blue, 2 grams of orange G., and 2 grams of oxalic acid. From this staining solution, the sections are rinsed in water, dehydrated rapidly with alcohols, cleared in xylol and mounted.

11. *Kolossow's osmic-pyrogallic-acid method* is used for the membranes and for the structure of the intercallated disks of cardiac muscle. The procedure is that followed by MacCallum (Anat. Anz., Bd. 13, p. 609, 1897; and Kolossow, Zeitschr. für Wiss. Mikros., Bd. 9, p. 38, 1892). It is applied to material fixed in alcoholic corrosive sublimate.

12. *Striated Muscle.*—Pieces of thin flat muscles, such as certain of the abdominal muscles or the diaphragm of mammals, are chosen preferably because their thinness allows more rapid fixation and because, in sectioning, the plane of section with reference to the direction of the fibres can be more easily controlled. Bands of these muscles, taken from a freshly killed animal, are pinned out flat and taut upon cork plates and placed in a moist chamber (a wet Petri-dish) for about 30 minutes, to allow complete relaxation before fixing. A drop of strong ammonia water may be added to the moisture of the chamber to hasten the relaxation. Then the cork plates with the muscle on them are inverted in a dish containing either saturated aqueous corrosive sublimate, Zenker's fluid, absolute alcohol or Gilson's fluid. After 5 or 10 minutes, the pieces of muscle, then stiffened somewhat, should be removed from the cork and placed in a closed vessel containing a copious amount of the fixing fluid, and set away for the length of time required by the fluid chosen. They are then washed as required by the fluid, and preserved in 70 per cent. alcohol. Blocks of the size needed for embedding are cut out with sides parallel and transverse to the direction of the fibres, embedded in either paraffin or celloidin, and thin sections stained lightly in hematoxylin. Most counterstains tend to block the detail and should be avoided. The structure may often be seen quite well in unstained sections, especially of material fixed in a fluid containing bichromate of potassium (Zenker's, for example) which itself leaves a brown tone.

13. *Mucins, mucous alveoli and goblet cells* are differentially stained by the application of Mayer's muchematin as employed by Bensley (Am. Jour, Anat., vol. 2, No. 1, p. 105, 1902). Paraffin sections are required, for, when applied to celloidin sections, the stain gives diffuse preparations.

14. *Kupffer's method* for sections of medullated axons is applied as directed in Hardesty's Neurological Technic, page 37. The application of 0.5 per cent. osmic acid to the straightened pieces of small nerve should be prolonged to at least 12 hours (the pieces removed from the bits of cardboard) and then

washed in water for an equal length of time. The washing from the fuchsin-absolute-alcohol solution may be done with absolute alcohol, and thus the pieces, already dehydrated, may be embedded in paraffin directly. If the thin sections do not show the axonic reticulum sufficiently strong, they may be again stained in acid fuchsin.

15. *Tigroid masses* (Nissl bodies) are demonstrated in the cell-bodies of neurones in more nearly their normal character by the application of erythrosin and toluidin or methylen blue than by the original Nissl method. Small blocks of central nervous system, and peripheral ganglia, are fixed in 96 per cent. alcohol or, equally well, in Van Gehuchten's fluid, sectioned in paraffin (5 to 10 micra), stained on the slide 10 to 15 minutes in 1 per cent. alcoholic erythrosin, washed briefly in 50 per cent. alcohol, rinsed in distilled water, and then stained in 1 per cent. aqueous toluidin blue for 10 to 15 minutes. The surplus stain is then drained off and the slide dipped a time of two through 1 per cent. aqueous potassium alum, dehydrated and further differentiated by passing rapidly through the graded alcohols, cleared in xylol and mounted. Methylen blue, made up according to the formula of Nissl, may be used instead of toluidin blue. In that case. the sections from which the stain has been drained are differentiated first in anilin-alcohol (10 per cent. of anilin oil in 95 per cent. alcohol) and further differentiated and cleared with oil of cajeput, which latter is rinsed off in xylol and the sections mounted.

16. *Cajal's method for neurofibrillæ* is the most satisfactory in every way of all the methods employed for this purpose. Of the three or four slightly different procedures suggested for this method, that calling for fixation in ammoniated alcohol gives the best results with mammalian tissue. The ventral horn cells of the spinal cord of the pig at term will be found especially fortunate, but good results may be obtained with tissue from the adult dog or cat. Descriptions of the method by its author, Cajal, may be found in Compt. rend. Soc. Biol., T. 56, No. 8, p. 368, 1904; also in Zeitschr. für Wiss. Mikros., Bd. 20, H. 4, p. 401, 1904; and in Bibliogr. Anat., T. 15, Fasc. 1, p. 1, 1905. The embedded tissue, preserved in alcohol, will keep for months in the block.

17. *The gold chlorid method* for nerve terminations and for sympathetic nerve plexuses. Löwit's method, slightly modified, is recommended for fresh tissue, especially for nerve endings on skeletal muscle and tendon and for the plexuses of Auerbach and Meissner. A piece of intestinal wall, or the bit of muscle into which a nerve twig is seen to enter, is cut out and placed in 10 per cent. aqueous formic acid for about 5 minutes, then transferred, without washing, to a 1 per cent. aqueous solution of gold chlorid for 15 to 20 minutes, then, again without washing, it is placed in a copious amount of 5 per cent. aqueous formic acid and put away in the dark for 24 hours, or more, till the gold is sufficiently reduced. Tissue stained in this way is usually studied by teasing on the slide. From the formic acid reducing bath, the pieces are washed in water and placed in glycerin where they will keep for years and from which pieces may be taken, teased on the slide and examined for good demonstrations

which, when found, are isolated and mounted in glycerin jelly. Of striated muscle, the plantar muscles, intercostals and abdominal muscles are recommended because of their lesser thickness and the ease with which nerve twigs may be traced into them.

The formic acid causes the tissue to take up water and swell considerably. This may be avoided by the use of tissue preserved in 10 per cent. formalin (4 per cent. formaldehyde). The procedure is practically the same with this tissue as the above except the time in the first formic acid bath and in the gold chlorid solution is doubled. Applied to formalin material, the method is less certain than when applied to fresh tissue, but when successful, after formalin, the results are more satisfactory. After either procedure, the tissue may be embedded and sectioned in paraffin.

Sihler's hematoxylin method for staining nerve terminations sometimes gives excellent pictures. However it requires rather severe maceration of the tissues and takes a longer time than gold chlorid. For detailed directions for any of the procedures, the operator is referred to the books.

18. *Methylen blue for nerve terminations* gives more delicate results than either of the above methods and is resorted to for terminations of sensory nerves in the periphery, especially upon blood-vessels, for terminations in the sense-organs and for terminations upon smooth muscle-fibres. It is also used for demonstrating the end-brushes of axones terminating about the cell-bodies of neurones in the central nervous system and in ganglia. When the animal is taken for the purpose it is safer to apply the stain both *intra vitam* by injection and later, to bits of tissue by *immersion*. The method, at best, is more uncertain than the gold chlorid and requires considerable practice. It is known as Ehrlich's methylen blue method, but the original procedure has been variously modified for different purposes. For smooth muscle, neuromuscluar spindles etc., the procedure given by Huber and De Witt (Jour. Comp. Neurol., vol. 7, p. 169, 1897) will be found trustworthy. For later directions, the operator is referred to the recent text-books and to the numerous papers of A. S. Dogiel, some of which papers are cited in the literature on the nervous tissues. When sections of the stained tissue are not required, Bethe's fixing bath may be omitted, and the tissue, taken from the ammonium picrate solution, may be teased in a mixture of equal parts saturated aqueous ammonium picrate and pure glycerin and then mounted in a glycerin jelly made up by using the ammonium picrate solution for its aqueous component.

19. *The Weigert method* for medullated nerve trunks in the central nervous system is given in all the text-books. Most of the more recent applications of the method involve Pal's modification of the procedure for differentiation, namely, the treatment of the celloidin sections with potassium permanganate, washing and subjecting them to the bleaching action of sulphurous acid, developed in a mixture of oxalic acid and potassium sulphite. Dilute sulphurous acid may be used direct. Instead of tissue fixed in Müller's fluid as regularly employed, tissue fixed and preserved in formalin may be used.

Small blocks of the latter are placed in 3.5 per cent. potassium bichromate and kept in a warm oven (35° C.) for 3 to 5 days, the fluid renewed at the end of the first day. Then they are washed in water, embedded in celloidin and the sections treated as after Müller's fluid. In either procedure, if, on trial the sections do not stain deeply enough in the hæmatoxylin, they are mordanted, for 10 to 20 minutes before staining, in a 1 per cent. aqueous solution of chromic acid, after which they are washed well and then subjected to the stain. Kulschitzky's hematoxylin gives a deeper stain than that of Weigert's formula and is to be preferred for most tissue.

THE SHARPENING AND CARE OF THE MICROTOME KNIFE.

Since failure to get preparations most suitable for study is frequently due to the poor condition of the sectioning knife, and since few students realize how delicate a piece of apparatus the microtome knife is or the nature of the cutting edge required, the following suggestions are offered for the sharpening and care of the knife.

In the first place it is folly to obtain a knife of other than the very best quality of steel, and one for general use in making celloidin sections should have a cutting edge of not less than 15 centimeters. The author has found the knives manufactured by Wilhelm Walb and by Carl Franck of very satisfactory quality.

The nature of the edge required is different from the ordinary razor edge in that it should be smoothly sharp. The razor used for shaving does excellent work when its edge is in the form of a fine saw. A hair drawn very lightly along such an edge may be seen to quiver perceptibly, though a slight increase of pressure will sever it quite readily. The edge of a microtome knife should be carried beyond the degree of sharpness of the ordinary razor edge. It should be smoother, and a hair drawn lightly along it should apparently not quiver at all. Especially in case of the larger sections, required in the study of microscopic organology, torn or "ribbed" surfaces produced by a "saw edge" of the knife render the preparations very unsatisfactory.

Constant vigilance is required to keep a knife in good condition. Especial care should be taken not to bring a needle or scalpel or any hard substance near its edge while using. The sections should always be handled with a soft brush. And it is an excellent rule always to strop the knife a minute or two both before and after using. The stropping after use is particularly advisable in that it cleans, dries and polishes the edge, making it safer against corrosion when put away. Placed in its special box, the knife should always be kept in a dry place and well away from possible fumes of acids. An apparently slight corrosion on the edge means serious injury to its cutting quality.

Honing.—When the knife becomes very dull or when nicks have been made in its edge, honing is necessary. This is a time-consuming process, and requires more thought and care than is often at first realized. When the knife

is very badly nicked, or when poor grinding has "rounded" its edge, it is wiser to send it to a reliable manufacturer of cutlery and have it ground down by machinery. But from the cutler, the edge will always have to be finished in the laboratory by light honing and by stropping in the right way. Small nicks and ordinary dulling are generally remedied in the laboratory.

For honing, the white, Arkansas stone, cut by the Pike Manufacturing Co., of Pike Station, N. J., is much superior to any the author has tried. This stone is of an exceptionally fine grit and, at the same time, being a very hard stone, it grinds the knife rapidly. Used with oil instead of water, a hone made

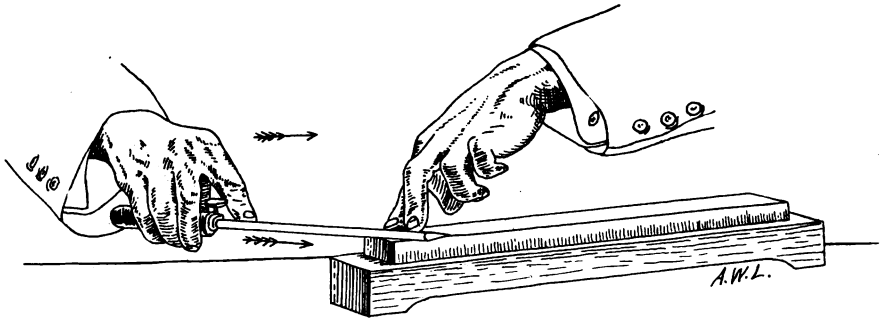

FIG. 25.

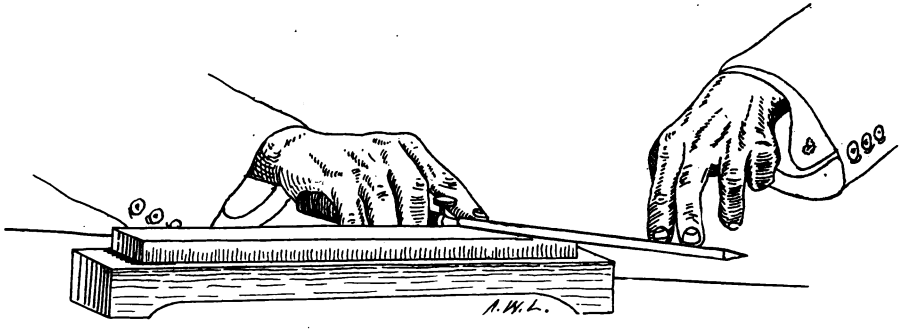

FIG. 26.

of this stone gives an edge fine enough to be transferred direct to the strop and thus it obviates the necessity of using first a coarse and then a fine-grained hone before the stropping. The hone should be 2½ inches wide and at least 12 inches long. Obviously, in honing knives with long cutting edges, the longer the hone the better. It should be fitted in a wooden casing as shown in the figures below, and the cover to this case should be kept on when the hone is not in use, to protect against dust and grit.

An excellent procedure in honing is illustrated in Figs. 25 to 28. The hone is laid flat on the table and, for right-handed operators, should be arranged diagonally with the end toward the left nearer the body of the operator. The

stroke in honing should always be edge foremost and the knife should pass somewhat diagonally along the hone, beginning at the toe of the blade and terminating at the heel. Figs. 25 and 26 show respectively the beginning and the termination of this stroke. For the return stroke, involving the reverse side of the blade, the knife is rotated with its back downward and resting upon the hone, at the same time being drawn toward the operator while the right hand,

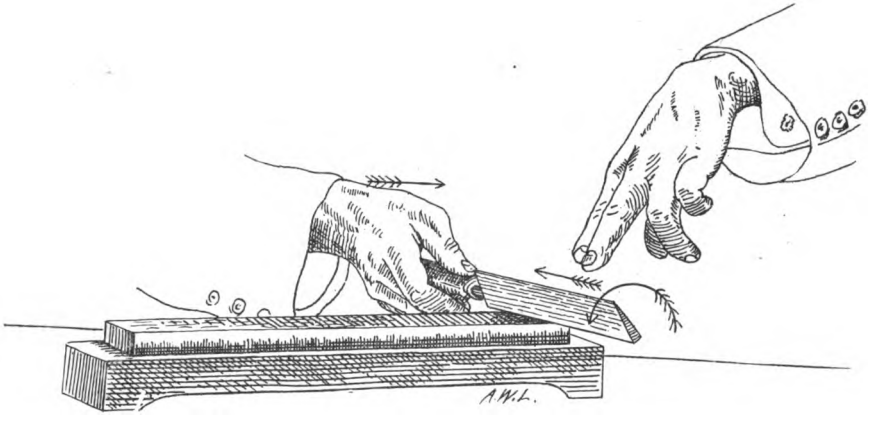

FIG. 27.

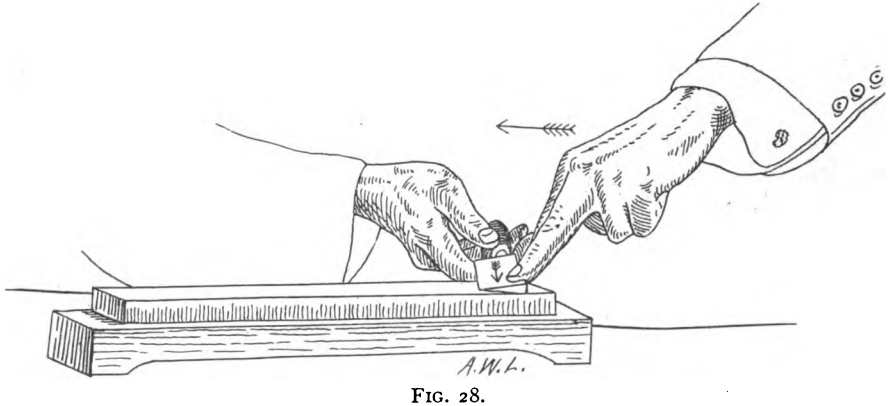

FIG. 28.

FIGS. 25 to 28.—Illustrating the position of the hone and the manipulation of the knife in honing.

holding the handle, approaches the left in order to again attain the diagonal direction of the stroke. The position for the stroke is then completed by letting down the side of the knife flat upon the surface of the hone and the stroke, again passing from toe to heel, edge foremost, is accomplished. Figs. 27 and 28 illustrate in part the manipulation for the second or return stroke. Repetition of the first stroke is made after repeating, at the right-hand end of

the hone, the rotation on its back and the drawing backward of the blade; here, however, the right hand is moved slightly away from the left to attain the diagonal position assumed when starting the stroke shown in Fig. 25. Then follows a repetition of the return stroke and so on, the two sides of the knife being ground alternately throughout the process. Especial care must be taken that, during each stroke, the blade is kept flat on the hone, with the edge parallel to its surface, to insure even grinding. A slip or a stroke upon the corner of the hone may incur a serious loss of time to grind away the resulting unevennesses in the edge. The holding of the blade flat and parallel upon the hone is rendered somewhat easier by the diagnoal position during the stroke and it may be further insured by the aid of the fingers of the left hand, used as shown in the cuts. The fingers of the left hand, however, are used chiefly to attain greater pressure upon the hone and thus more rapid grinding. Care, of course, must be taken that the pressure exerted by the left hand balances that of the right especially during portions of the stroke when the left hand is not immediately over the hone. The aid of the left hand should be renounced toward the end of the required period of honing.

The advantages of the above procedure in honing lie in the rapidity and skill possible with which an evenly ground edge may be obtained. With a little practice of the strokes, giving special attention to the separate movements, a speed of two strokes per second may be acquired.

Most microtome knives are made with one side plane and the other side concave or "hollow-ground" and, therefore, in honing, the stroke grinding the concave side cuts away the steel near the edge more rapidly than the stroke grinding the plane side. Only in case of very large nicks in the edge should the knife be ground for even a short period on the concave side only. The procedure described above, grinding each side alternately, is always safer in securing an even grinding of the edge.

On request, manufacturers send detachable metal backs to be clamped upon the plane side of knives while honing so that the edge is tilted toward the surface of the stone, and may be ground down more rapidly. These should never be used except when great haste is necessary and the knife is very dull, for the time saved then will have to be given later in bringing the knife to its proper condition. The detachable back results, of course, in an edge beveled on both sides. The more nearly the line of the edge coincides with the plane of the lower or plane surface of the knife, the better, for in making sections, the knife is used with the plane side next the block of tissue and an edge beveled on this side is obviously undesirable.

When the nicks have disappeared and it has become evident that on the plane side of the knife the surface of the stone comes in actual contact with the edge throughout the stroke, then the pressure upon the knife should be decreased. It should then be honed lightly and very evenly till no "wire edge" is manifest and till it will readily cut a hair throughout its length. Then the knife is ready for stropping.

Stropping.—The best form of strop for microtome knives does not seem to be obtainable on the general market. It may be made in the laboratory by procuring from the leather dealer a piece of the best quality of calf-skin, soft, dry and smooth of surface, 3 inches wide and about 20 inches long. Then a piece of inch-thick board of some solid wood is cut 3 inches wide and 26 inches long, and one surface dressed till it is perfectly plane and smooth. Then, beginning at one end, melted glue is evenly brushed over 20 inches of the side especially dressed and the piece of calf-skin, with the grain or epidermal side outward, is placed in position upon the glue, care being taken that no glue gets upon the outward surface of the leather. The strop should now be placed

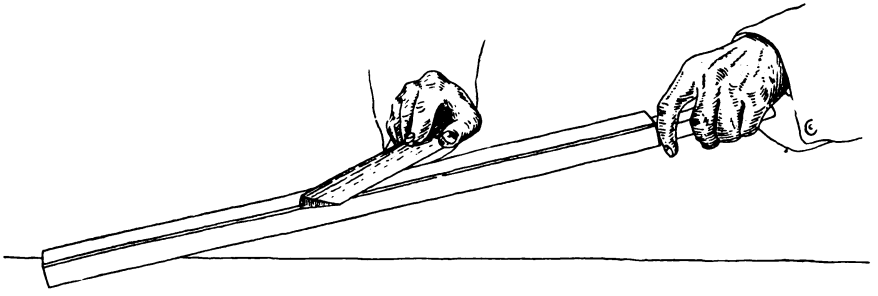

FIG. 29.

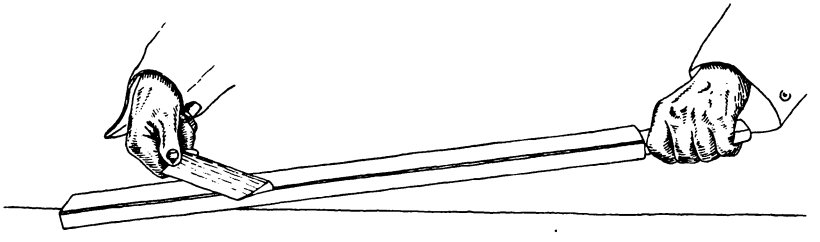

FIG. 30.

FIGS. 29 and 30.—Showing the form of strop desirable and the direction of the two strokes which may be employed in stropping.

between two strong boards and the whole clamped firmly in a vise and held there for 24 hours. The surface of the clamping board which presses upon the leather should, of course, be clean and likewise plane and perfectly smooth. After the glue has "set" well, the strop may be removed from the vise, the edges trimmed, drops of excessive glue removed, and the extra 6 inches of the length of the broad may be dressed into a handle for gripping the strop. The strop shown in Figs 29. and 30 was made in this way. It, however, has a leather surface 26 inches long, a desirable difference from the one described above but not essential, and it is often difficult to obtain a strip of calf-skin of even quality throughout a length of 26 inches. This form of strop is superior

to the common forms because of its firm surface. Any form of strop with bending or even yielding surface tends to close upon the edge of the knife, rounding it off, instead of giving the truly keen edge so desirable in a microtome knife.

The process of stropping is quite similar to that of honing, except the stroke is always made with the back of the blade passing foremost and one end of the strop (the handle) is held in the left hand while the other end rests upon the table. The rotating and drawing backward on its back of the blade at the end of each stroke are identical with the similar movement in honing. The stroke shown in the figures is diagonal along the strop and passing from toe to heel of the blade. In stropping, the left hand aids the right in giving the required diagonal direction to the stroke by moving the handle of the strop back and forth as the downward and upward strokes are made. Some operators make the stropping stroke passing from heel to toe, and claim that it is more efficient if the stroke in honing was made from toe to heel. This claim, however, is questionable from the fact that whatever teeth made by the hone with the knife passing from toe to heel, edge foremost, are probably obliterated more rapidly by a stropping stroke passing from toe to heel but with the back of the knife foremost and the stroke diagonal in the *opposite* direction to that used in honing the given side of the knife.

When tests with hairs show that the knife has a smooth and truly keen edge, it may be pronounced in good condition. The condition of the edge may be judged by examining it under the microscope, using mediumly low magnification, if one has become sufficiently experienced as to the appearance of edges in different conditions as seen under the microscope Place a fold of paper under the blade to keep the edge from touching the stage of the microscope and draw the blade across slowly, keeping the edge in the field of vision. The smaller and more even the teeth shown by the microscope, the more nearly smooth is the edge, and the better its condition.

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